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SICKLE CELL ANEMIA*

BY WILLIAM Z. FRADKIN, A.B., M.D., AND LEO S. SCHWARTZ, M.D., F.A.C.S., BROOKLYN, N. Y.

SICKLE cell anemia is a hereditary blood dyserasia with an unusual extensive symptomatology. Because of the latter surgeons often mistake this disease for an acute abdominal condition, or clinicians for acute hemolytic icterus or syphilis. It is only by the accidental discovery of sickle cells in the routine examination of the blood smear, that the diagnosis is made. In this paper it is our intention to review the literature and report a case of sickle cell anemia with rapid improvement, following laparotomy and transfusion.

Sickle cell anemia was first discovered by Herrick in 1910. Up to the year 1923 only four reports of similar cases appeared in the literature. Mason was the first to use the term "sickle cell anemia" in his report of the fourth case. Sydenstricker was the first to really stimulate interest in this disease. His papers read before the members of the Southern Medical Association and the American Medical Association in 1923 and 1924 were the most accurate and scientific presentations of the time, and established sickle cell anemia as a definite clinical entity with a characteristic blood picture and a definite pathology.

Sydenstricker divided the disease into the active and latent phases according to the condition of the blood and the severity of the symptoms. In the active phase, sickling of the erythrocytes is present, whereas in the latent phase, sickling is absent at first, but can be seen after the fresh blood specimen is four hours old. In the active phase anemia is pronounced and the pa-

*From The Gynecological Service of Dr. Schwartz of The Jewish Hospital of Brooklyn. Received for publication, September 24, 1929.

tient often complains of dyspnea, palpitation, and weakness. Pain, arthritic and muscular, without evidences of inflammation, is constant. Epigastric pain after eating and recurrent attacks of epigastric and left hypochondriac pain have been present in most cases, and vomiting may occur. There is fever of low grade, and night sweats are common. The patient is poorly developed and poorly nourished. The sclerae present a characteristic greenish discoloration quite different from the muddy color commonly found in negroes and the yellow of biliary obstruction. The mucous membranes are pale, there is hypertrophy of the tonsils, and the superficial lymph nodes are uniformly enlarged. The lungs frequently show evidence of pulmonary congestion. The heart is enlarged to the left with a systolic murmur at the mitral area. The heart rate is accelerated and the blood pressure is low. The liver is constantly enlarged while the spleen is hardly ever felt. The extremities may show ulcers or the scars of ulcers, and these frequently lead the physician to a temporary diagnosis of lues. The blood however shows elongated sickle cell and oat-shaped poikilocytes in numbers varying from 5 to 40 per cent. When the blood is sealed under a coverslip, bizarre and crescentic forms appear within a few hours, and increase in number until after twenty-four to thirty-six hours from 40 to 90 per cent of the cells have assumed sickle forms. The reticulocytes are frequently increased, the percentages varying from 6 to 30 per cent.

In the latent phase patients present no symptoms of anemia but give a history of rheumatic attacks, pain in the epigastrium and left hypochondrium and periods of weakness and dyspnea which have been separated by years of normal health. Fresh blood preparations may show a few or no sickle cells, but upon standing many cells assume the sickle form.

Sydenstricker was also the first to describe in detail the pathologic findings in this condition. The most important findings are revealed in the study of the blood, liver, spleen, and bone marrow. The blood shows the characteristic fusiform and sickle shaped erythrocytes. The liver is hypertrophied and shows evidences of cloudy swelling with iron-free pigment in both the liver cells and the Kupffer cells. The spleen is firm and on section the cut surface is very dark red with no visible lymphoid nodules. Microscopically the spleen seems overfilled with blood, the trabeculae are prominent, and the small malpighian bodies are surrounded by areas of intense congestion. The sinuses and the spaces of the pulp are engorged with blood. The endothelial cells of the sinuses are heavily laden with brown iron-free pigment. The bone marrow is usually abundant, bright red, and thin in consistency. The reticular spaces are filled with erythrocytes intimately mingled with leucocytic constituents of the marrow. Large clusters of sickle shaped erythrocytes may be seen between the capillaries.

Huck in his experimental studies of sickle cell anemia confirmed the work of Sydenstricker and added the following points:

1. If the cells of patients are placed in physiologic salt solution, or in isotonic sodium chloride containing 1.25 per cent sodium citrate, or in oxalated whole blood, and fresh preparations made from these suspensions, the red cells, on standing, will take on bizarre forms.

2. If normal red cells of the same blood group as the patients are washed in isotonic salt solution and suspended in the patient's serum, the normal cells will remain unchanged.

3. Preparations of blood from cases of pernicious anemia, secondary anemia, myeloid and lymphoid leucemia, congenital hemolytic jaundice, and Banti's disease, show no change in their morphology after standing for one week.

4. Intravenous injection into rabbits of a 50 per cent suspension of washed red cells of a patient with severe symptoms of the disease provoked no visible abnormality in the blood of these animals.

5. Repeated blood cultures are negative in patients with sickle cell anemia.

Graham reported a case of sickle cell anemia with necropsy and pointed out that the red cells of the tissues fixed in formaldehyde were all sickle cells, but those of the tissues fixed in Zenker's solution were normal in shape.

Moser and Shaw, and Anderson, in their report of one case each in Northern negroes, showed that this disease was not peculiar to the Southern or Tropical negroes.

Sickle cell anemia may be complicated by gallstones, in which case the differential diagnosis is extremely difficult, clinically, because of the presence of gall bladder symptoms similar to that of gall bladder pathology. Such a case was reported by Hamilton.

Cooley and Lee found sickle cells present in the bloods of 7½ per cent of the 400 patients admitted to their clinic during a period of ten months. From their studies of the phenomena of sickling they conclude the following:

1. In preparations kept at incubator temperature the sickle cells disappear rapidly from the blood.

2. The cells of one of the patients with sickle cell anemia were rapidly hemolyzed at incubator temperature in their own serums, and in serums from normal bloods, while the serum of this patient was not hemolytic for normal cells.

Alden in his report of two cases emphasized the fact that sickle cell anemia may be confused with tertiary syphilis due to the frequent presence of leg ulcers. In his second case the pain in the abdomen and sudden distention were thought to be due to an acute appendicitis, but in all probability were due to a splenic hemorrhage.

Josephs examined 14 samples of blood from patients with sickle cell anemia by thoroughly washing the red cells in salt solution, and succeeded in removing an unknown substance which is responsible for the sickle cell formation.

In a paper on the origin and fate of sickle shaped red blood cells, Levy described peculiar changes in the red cells. Embryonic red blood cells and normoblasts divide or extrude their nuclei. The resultant cells project pseudopodia and assume bizarre shapes. Heat hastens the change and cold inhibits. Some of the abnormal red blood cells retract their pseudopodia and return to the parent form while others retain their bizarre shapes permanently. Sickle cells kept at body temperature eventually disintegrate by fragmentation.

Hahn and Gillespie were the first to attempt splenectomy for the treatment of active sickle cell anemia. The patient showed evidences of great blood de-

struction with a rapid drop in the red cell count. A splenectomy was followed by marked clinical improvement with a rapid return of the red cell count to almost normal figures. These authors also report a series of experiments in which various gases were passed through a chamber containing a suspension of a patient's red cells. They showed that the red corpuscles of persons with the "sickle cell trait" are transformed into sickle cells in vitro as a result of asphyxia. The transformation takes place when the oxygen tension falls below a partial pressure of 45 mm. of mercury provided the hydrogen-ion concentration is within certain limits, probably always on the acid side of P_H

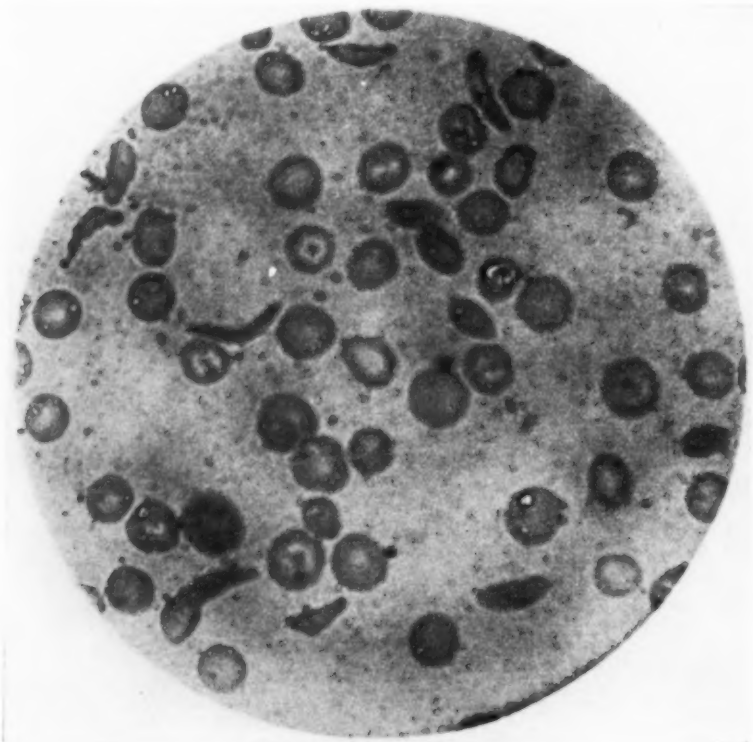

Fig. 1.—Stained blood smear.

7.4. Oxygen and carbon monoxide induced restoration of the discoid form. They are of the opinion that sickle cell formation in vivo is probably induced or increased by anoxemia.

Hein, Gordon, McCalla and Thorne placed red cells of their patient with sickle cell anemia in sera, of his own mother, of a normal negro, of a patient with pernicious anemia, of a patient with acquired type of hemolytic icterus, of a patient with obstructive jaundice, and noted the development of the typical deformity in the cells. Conversely, the red cells of these people when placed in the serum of the affected negro boy showed no sickling.

Stewart observed a case of sickle cell anemia in a child six years of age, neither of the parents were of direct negro descent. It is the only case on

record in an apparently white child of Cuban descent. The spleen was observed by the author in its transition from a hypertrophic to an atrophic organ. A splenectomy was performed with a seemingly rapid but uneventful recovery.

Bell, Kotte and Mitchell reported a case of sickle cell anemia in a colored boy aged eighteen months where splenectomy did not affect the sickle cell picture nor lessen the existing marked anemia. Cooley and Lee suggested an explanation for the latter by stating that a supernumerary spleen might have been overlooked at the operation.

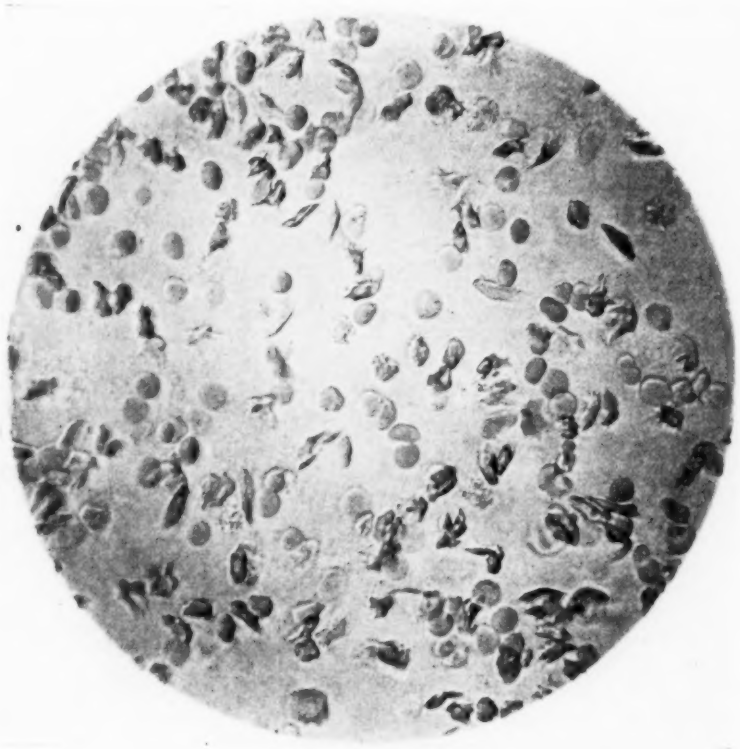

Fig. 2.—Sealed preparation of blood after twenty-four hours.

Wollstein and Kreidel reported two cases of sickle cell anemia in colored children whose cause of death was apparently due to the anemia itself, for at autopsy no other cause of death was demonstrable.

It is interesting to note that Rich in a study of the sections from 5000 consecutive autopsies was able to demonstrate a characteristic splenic lesion in 62 cases. The specific histologic appearance consisted of a pronounced malformation of the sinuses immediately about the malpighian bodies leading to the formation of pools of blood partially or completely surrounding the malpighian bodies. Sickle cells were found in the blood in the histologic sections in every case. Immediate relatives of these 62 autopsied individuals were examined by Josephs and in each case the sickle cell trait was found.

Bennett was able to recognize sickle cell anemia from necropsy material. He crushed blood clot from tissues hardened in 10 per cent formaldehyde, in physiologic solution of sodium chloride, and made smear preparations from the resultant suspension. Microscopic examination of these smears showed the typical sickle cells. The lapse of time after fixation seems to be of no importance in the amount or character of the red cell distortion.

REPORT OF CASE

E. R., a colored female, aged twenty-six, married, and residing in Brooklyn for the past three years, but born and reared in South Carolina, came to the

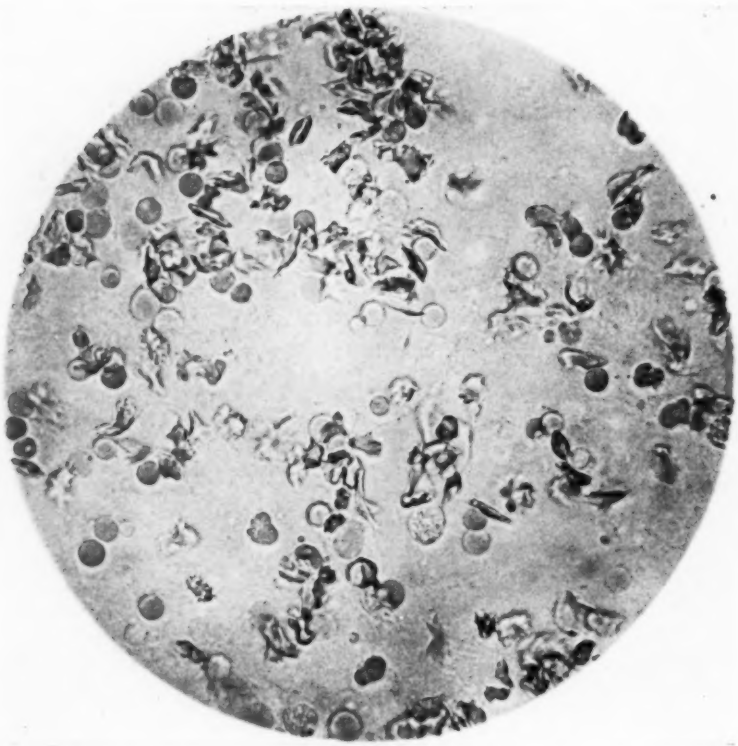

Fig. 3.—The same blood preparation after forty-eight hours.

Medical Clinic of the Out-Patient Department of the Jewish Hospital on March 13, 1929, complaining of pain in the abdomen.

A medical condition being ruled out, the patient was referred to the Gynecological Clinic. On examination a diagnosis of acute adnexitis was made and the patient was admitted to the Gynecological Service of the same hospital on April 4, 1929.

Her chief complaint was pain in the abdomen. The family history was negative. During her childhood the patient had measles, whooping cough, chicken pox, and smallpox. At the age of nine she had "rheumatism" with recurrent attacks of pains and fever up to the age of fifteen. Since the age

of fifteen, the patient states that she feels pains in the joints and muscles occasionally. Had malaria at the age of thirteen. Denies venereal infection. Has a negative surgical history. Marital history irrelevant. Menstrual history negative. Patient has had dyspnea and palpitation on exertion for many years. Has always had frequent spells of dizziness. Except for the pains in the extremities and back, the patient was feeling generally quite well up to the present illness.

The present illness dates back to ten weeks ago when the patient was seized with dull aching pain localized in the lower abdomen, more severe in the right lower quadrant. There was no nausea or vomiting present. Patient had fever and a yellowish white vaginal discharge since the beginning of the illness. On the day of admission the patient also developed some epigastric pain, with tenderness and rigidity over the right and left upper quadrants, especially on the right side. Appetite was fair with only slight distress after meals. Constipation was present. Slight dysuria but no frequency.

Physical examination revealed a colored female well developed but poorly nourished, anemic, and appearing acutely ill with a temperature of 102° F. Eyes were prominent but showed no other signs of exophthalmic goiter. Right eye showed a corneal scar adherent to the iris. Both sclerae were greenish yellow in color. Pupils were equal and reacted to light and accommodation. The palpebral conjunctivae were very pale. Nose and ears were negative. The mucous membrane of the mouth was very pale, tongue slightly coated, teeth in fair condition, tonsils submerged but cryptic. Neck revealed small palpable cervical lymph nodes. The chest showed evidences of early rickets. The lymph nodes in the axillary fossae were palpable.

Examination of the heart revealed a soft systolic murmur at the apex and along the left border with a loud systolic over the aortic and pulmonic areas.

The lungs revealed occasional subcrepitant râles at extreme right base posteriorly.

The abdomen was tense, doughy, with resistance and tenderness over the right upper quadrant, and somewhat over the left hypocondrium. There was also some tenderness at McBurney's area. The liver was palpable, 3 cm. below the right costal margin. The spleen was not felt. Kidneys were not felt.

The upper extremities were negative. The tibiae of both lower extremities were prominent and showed large scarred areas over the middle third of the anterior surfaces. Reflexes were apparently normal.

LABORATORY FINDINGS

Blood Picture—

Hemoglobin (Dare)	36 per cent
Red blood cells	2,300,000
Color index	0.78
Bleeding time	2 minutes 45 seconds
Coagulation time	6 minutes 0 seconds
Platelets	120,000
Total white blood cells	12,400
Polymorphonuclears	53 per cent

Laboratory Findings

Blood Picture—Cont'd

Eosinophiles	1 per cent
Basophiles	0.75 per cent
Lymphocytes	38.0
Turk cells	1.0
Monocytes	5.0
Promyelocytes	0.50
Neutrophilic myelocytes	0.75

There were 2 nucleated red blood cells in the 400 cells counted
Marked oligochromasia
Occasional stippled red blood cells
About 8 per cent of stained red blood cells were sickle-shaped (Fig. 1.)
Fresh preparation shows 25 per cent sickling and 98 per cent sickling after twenty-four hours. All red blood cells rounded after seventy-two hours (Figs. 2 and 3.)
Marked anisocytosis
Poikilocytosis
Macrocytosis and polychromasia
1 per cent normoblasts per 100 white blood cells
14 per cent reticulation
Supravital staining showed one mononuclear small sized white cell which has phagocytosed a red blood cell.
Red blood cells showed increased resistance
Hemolysis began at 0.34 per cent saline and was still incomplete at 0.28 per cent saline

Sedimentation time	18 minutes
Wassermann	Negative
Kahn	Negative
Bilirubin: Direct	Negative
Indirect	1 unit
Icterus index	12
Sugar	115 mg. per 100 c.c.
Urea	7.8 mg. per 100 c.c.
Creatinine	1.6 mg. per 100 c.c.
Cholesterol	238 mg. per 100 c.c.
Chlorides	625 mg. per 100 c.c.
Calcium	9.2 mg. per 100 c.c.

Renal Function (Phenolphthalein)—

First hour	56 per cent
Second hour	19 per cent
Total renal function	75 per cent

Urine—

Specific gravity	1.010
Reaction	Acid
Albumin	Faint trace
Sugar	0
Microscopic	Occasional white blood cells
Urobilinogen	1500 dilution units
Urobilin	2700 dilution units

Stool—

Bile	Present
Blood	Negative guaiac
No ova or parasites found	
Urobilinogen	4800 dilution units
Urobilin	14,400 dilution units

Gastric Analysis—

Free hydrochloric acid	2 per cent
Total hydrochloric acid	3 per cent
Lactic acid	Negative
Microscopic	Negative

Vaginal Smear — Negative for gonococci

Cervical Smear — Negative for gonococci

Vaginal examination revealed the uterus to the left, retroverted, and a fixed insensitive cystic mass in the right and posterior fornices.

The patient's temperature fluctuated from 100° to 103° F. seldom remaining low for a whole day at a time.

The patient's mother, brother, and two sisters were examined and none of them showed a condition similar to that of the patient. Fresh and old blood preparations both dry and wet showed no active or latent sickling of the red blood cells.

The patient did not respond to medication and nonspecific protein injections. A surgical consultation was requested in order to rule out a possible acute abdominal condition. A diagnosis of acute cholecystitis was made, and the patient was therefore transferred to the surgical service. On opening the abdomen the liver was found adherent to the abdominal wall. The peritoneum was studded with tubercles varying in size from a millet seed to a pea. All of the small intestine was covered with minute tubercles. Many of the loops were adherent. The gall bladder was negative. The spleen was small, covered with tubercles and adherent to the anterior abdominal wall. The pelvis was found walled off by adhesions, and the tubes were probably the site of tuberculous invasion. The mesenteric lymph nodes of both small and large intestines were enlarged to the size of a walnut.

Microscopic examination of a mesenteric lymph node revealed active tuberculosis.

The patient became very weak following laparotomy. A blood count revealed 2,200,000 erythrocytes and 34 per cent hemoglobin (Dare). An intravenous transfusion of 500 c.c. of blood was given on May 15, 1929, with prompt benefit. The general condition of the patient began to improve steadily, the temperature dropped to normal, the abdominal tenderness and rigidity disappeared, the abdominal wound healed rapidly, and the patient was discharged on June 2, 1929, nineteen days following operation.

COMMENT

This patient complained of pain in the lower abdomen, fever and vaginal discharge. After admission she also developed epigastric pain with tenderness and rigidity over the right and left upper quadrants. It was thought that the patient had a subacute pelvic peritonitis in addition to the already existing adnexitis. The sudden onset of upper abdominal symptomatology however made a diagnosis of an acute cholecystitis possible. Although a diagnosis of sickle cell anemia was made during the first week of the patient's stay in the hospital, its significance was not realized until after operation. Pain, tenderness, and even rigidity over the gall bladder region are very common symptoms and signs in cases of sickle cell anemia.

Patients with sickle cell anemia are very susceptible to tuberculous infections, and in this case a diagnosis of tuberculous adnexitis should have been considered. In fact, over 50 per cent of the reported cases of sickle cell anemia succumbed to tuberculosis. This patient had an apparently negative chest on x-ray examination. Dolgopol and Stitt found the sickle cell phenomenon in 5.2 per cent of the 77 tuberculous patients they examined. Tuberculosis can-

not therefore be considered as an etiologic factor in the development of sicklemia, but rather a complication as a result of the lowered resistance of the patient.

Clinically, sickle cell anemia closely resembles familial hemolytic jaundice. The latter condition however shows an increased fragility of the red blood cells, an enlarged spleen and absence of leg ulcers or scars, joint pains and crises of abdominal pain.

It is interesting to note that in this case the direct Van den Bergh was negative, the indirect only 1 unit, while the icterus index was 12 with a marked increase in the urobilin and urobilinogen of the urine and stool.

Upon careful questioning of the patient regarding the scars over her legs she stated that she had blisters over the legs as the result of sitting barelegged in front of the fireplace during the winter months of her childhood, and that those blisters broke leaving scars over her legs. This is interesting for there is no such explanation as far as we know in the literature.

CONCLUSIONS

1. A complete review of the literature of sickle cell anemia is given.

2. A case of sickle cell anemia complicated by mesenteric tuberculosis is reported with improvement following laparotomy and transfusion.

3. A diagnosis of an acute surgical abdomen in a negro patient is hazardous without a previous search for sickle cells in the blood smear.

4. Emphasis is directed to the fact that most patients with sickle cell anemia are susceptible to tuberculosis probably because of their lowered resistance to infection.

5. A rather simple explanation is given for the leg ulcers or scars so often encountered in patients with sickle cell anemia.

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SPONTANEOUS MENINGEAL HEMORRHAGE*

By Frederick H. Lamb, M.D., Davenport, Iowa

CASE 1.—About ten years ago with one of my confreres, I saw a twelve-year-old girl, who had been seized sixteen hours previously with a sudden sharp, excruciating pain in the head. She had been playing the piano, when she stopped suddenly, ran to her mother with her hands clasped tightly to her head. She screamed with pain, which seemed quite unendurable, soon became irrational, vomited, and tossed about on the bed, crying out, and moaning in agony. Presently she lost consciousness completely.

When seen fourteen hours later, the child was profoundly comatose. Her pulse was 60, temperature 100.5° F., respirations slow and labored. The pupils were equal and dilated, reacted incompletely to strong light; there was a spontaneous lateral nystagmus; the conjunctivae were injected. She had had involuntary micturition and defecation, and a brisk epistaxis lasting about twenty minutes. There was a definite general rigidity of the body. The leucocyte count was 14,200. The urine contained a trace of albumin, but no sugar, acetone, diacetic acid, nor casts.

There was nothing significant in the child's family, nor her own medical history. No one knew of her having had any injury to the head.

A lumbar puncture yielded 60 c.c. of uniformly bloody fluid under greatly increased pressure. On standing a half hour, the red cells had settled in the test tube so that the upper fourth of the contents was water color, and at the end of twenty-four hours at 8° C., the cells had settled down to about one-tenth the total volume, with no sign of clotting. Smears and cultures were bacteria free.

Following the spinal drainage, the patient's general condition began to improve. After a second withdrawal of fluid, her condition warranted the hope of recovery. She improved steadily, and at the end of two weeks, seemed to have quite fully recovered from her experience. She is now a healthful young woman.

This was my introduction to that condition known, perhaps for want of a more significant name, as spontaneous meningeal hemorrhage (although at that time I believed it to be the clinical picture and symptom complex of non-traumatic pachymeningitis hemorrhagica interna). That there is a similarity in these two conditions might be deduced from a superficial scanning of the literature; yet a more careful study will bring out distinctions, which deserve a much more general appreciation.

INCIDENCE

It is difficult sometimes to know whether a disease is occurring more frequently or whether it is being recognized more commonly. Certainly, as Josephine B. Neal[1] says, there are but brief references to spontaneous meningeal hemorrhage in English, although in France and Sweden, a rather extensive literature has been developed.

My experience with 7 proved cases, and 2 probable cases, within a ten year period leads me to think that the condition is overlooked more fre-

*Read before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6, and 8, 1929.

quently than it is diagnosed. It has, furthermore, pointed out the need of differentiating this condition from certain other intracranial hemorrhagic conditions.

PATHOLOGY

Let us say at once, that we are not concerned with those hemorrhages occurring as a complication of such conditions as pyogenic or tuberculous meningitis, arthritis, septicemia, pertussis, smallpox, anthrax, nephritis-uremia, eclampsia, insolation, caisson disease or aviator's sickness. Neither are we concerned with the hemorrhagic diatheses: scurvy, purpura, hemophilia, nor leucemia, and only indirectly with lead, alcohol, syphilis, and arteriosclerosis, in so far as they may be contributing factors in individual cases. In most instances of spontaneous meningeal hemorrhage, these factors have no bearing whatever.

We cannot, however, dispose so readily of the sequellae of cranial trauma, nor of congenital aneurysms near the circle of Willis as etiologic factors. We are directly concerned in the differential diagnosis with subdural hematoma, in its various stages of development; also, with the similar if not identical condition, pseudomembranous pachymeningitis hemorrhagica interna.

To review briefly the latter condition on account of its close relationship to the subject of this paper, there is a growing belief that chronic subdural hemorrhage, and the pachymeningitis hemorrhagica interna of Virchow[2] are one and the same.[3] It is interesting to note that chronic subdural hemorrhage is of venous origin, thereby differing from all other forms of intracranial hemorrhage, which are generally the result of arterial rupture.[4] The pathologic picture as found at operation or autopsy—and certain of the clinical symptoms, are admirably explained by the mechanism of forming the subdural hematoma. Accoring to Holmes[4] and others, the hemorrhage is supposed to result from the tearing of small veins which enter the tributaries of the superior longitudinal sinus. At the point at which the superior cerebral veins enter the sinus, the arachnoid is firmly adherent to the dura mater. This anchorage evidently protects the large veins, and the sinus itself, while the small vessels are stretched and torn by displacement of the brain, resulting from traumatic force. The ultimate result is a more or less constant seepage of blood with organization of the clot more or less in keeping with the age of hematoma.

DIFFERENTIAL DIAGNOSIS

From a clinical standpoint, it is desirable to recognize the extradural hemorrhage, due for example to rupture of the middle meningeal artery, and an encysted subdural hematoma referred to above, following more or less remotely a blow on the head. Direct surgical relief is possible in both conditions. It is also desirable to distinguish between these two conditions, as a group, and the primary subarachnoid hemorrhage due, for example, to the rupture of a circle Willis aneurysm, or the primary cerebral hemorrhage with or without extension into the subarachnoid space. Neither of the latter conditions is amenable to surgery.

Briefly stated, the clinical syndrome of spontaneous meningeal hemorrhage is the picture of severe meningeal irritation with a "brutal onset," and without obvious cause; the uniformly bloody spinal fluid under increased pressure, and usually prompt relief of symptoms following removal of fluid.

On the other hand, the symptomatology of chronic subdural hemorrhage is extremely variable. According to Holmes, there is the history of injury, the gradual onset of headache, sleeplessness, forgetfulness or vertigo. There may be a general lowering of efficiency, general stupidity, irritability, often a thick aphasic speech. The second stage is characterized by a sudden aggravation of symptoms. The headache becomes worse, the drowsiness passes into stupor, and periods of consciousness alternate with periods of unconsciousness. Finally a moderate hemiparesis develops due to unilateral cerebral compression, although a frank hemiplegia is rare.

The symptoms of intracerebral or capsular hemorrhage are too well known to reiterate here. The association is between coma and paralysis, instead of coma and paresis, and the history of the two conditions is unlike.

SPINAL FLUID

In connection with the clinical symptoms of spontaneous meningeal hemorrhage, the most valuable single finding is the character of the cerebrospinal fluid. A uniform and decidedly bloody fluid under pressure is evidence of gross hemorrhage into the subarachnoid space. It is the cardinal sign of spontaneous meningeal hemorrhage. It is very much less likely to be found in either the typical case of extra, and subdural hemorhage, or intracerebral hemorrhage. Conceivably both of these conditions may advance to the point of blood escaping into the subdural space, and the finding of a few erythrocytes in microscopic examination, or presence of xanthochromia is not infrequently observed. But the presence of a large amount (7 per cent, or more) of blood in fluid under greatly increased pressure is a strong point in favor of meningeal hemorrhage. Furthermore, if the fluid be recovered within twelve to twenty-four hours after the onset of symptoms, it will be observed that on standing, the supernatant fluid is water color. Succeeding withdrawals of fluid will show an increasing xanthachromia, with or without a diminution in the number of red cells.

It is worth while noting also that the fluid, in spite of its large blood content, shows no tendency to clotting. This is, indeed, quite the opposite of the prompt and firm clotting which takes place in an admixture of spinal fluid and blood of traumatic origin in performing the lumbar puncture.

In this connection, I have observed the effect of mixing in vitro, normal blood and normal spinal fluid from the same and different individuals, on the type of clot, and the clotting time. Briefly stated, the admixture will produce a firm clot in the same time as the blood alone in proportions up to 1 part by volume of whole blood to 7.5 parts of spinal fluid. Greater dilution of the blood will cause an increase in clotting time of the mixture, but result in a firm uniform clot up to 1 part of blood to 22 parts of spinal fluid, at which point the clotting will be delayed about fifteen minutes. A still greater dilu-

tion of the blood results in a partial clotting of the mixture up to 1 part of blood to about 35 of fluid with a corresponding but not equal lengthening of the time.

The point is that even though the amount of blood in the fluid obtained in spontaneous meningeal hemorrhage, be much greater than that required to clot a mixture of normal blood and fluid in vitro, no clotting takes place.

Additional spinal fluid findings will be referred to later.

REPORT OF CASES

CASE 2.—Mr. F. J., white, carpenter, aged forty-one, was seen with Dr. Haller at St. Luke's Hospital, Davenport, Iowa, May 17, 1922.

Present Illness.—While shingling the roof of a house, the patient was seized with a sudden excruciating pain in the head. He had to be helped to the ground, and was unable to walk home. There was no history at that time or previously of any injury to the head. He was a chronic alcoholic. On entering the hospital, he complained bitterly of headache, was very impatient and resentful, that he had had no relief.

His temperature varied from 98.0 to 99.8° F.; pulse 50 to 78; respirations 20 to 24; R.B.C. 5,380,000; W.B.C. 9,400; Hgb. 93 per cent; coagulation four minutes; Wassermann negative; urine: albumin +; casts: occasional hyaline. X-ray of head: No sign of fracture. Pupils: Slightly dilated, equal and regular; direct and consensual light reflexes and to distance are prompt. Eye grounds: Slight clouding of the discs.

Withdrawal of cerebrospinal fluid gave prompt relief of symptoms. Cerebrospinal fluid Wassermann negative.

SPINAL FLUID

DATE	AMOUNT	PRESSURE MILLIMETERS OF MERCURY	ERYTHROCYTES	LEUCOCYTES	CLOT	SUPERNATANT FLUID
5/19/22	50 c.c.	+++	+++		0	Water color
5/20/22	25 c.c.	22	160,000	900	0	Amber tint
5/22/29	25 c.c.	17	90,000	980	0	Light amber
5/24/29	20 c.c.	14	10,000	150	0	Amber

The patient left the hospital at the end of ten days seeming to have fully recovered.

While at work June 1, 1922, he had a second attack similar in all respects to the attack two weeks previously. He was again admitted to the hospital with symptoms, signs, and course of the illness practically parallel to his first admission. Relief of symptoms followed the third lumbar puncture.

SPINAL FLUID

DATE	AMOUNT	PRESSURE MILLIMETERS OF MERCURY	ERYTHROCYTES	LEUCOCYTES	CLOT	SUPERNATANT FLUID
6/1/22	35 c.c.	22	210,000		0	Amber
6/3/22	25 c.c.				0	"
6/4/22	25 c.c.				0	"
6/5/22	20 c.c.				0	"
6/6/22	25 c.c.	15	80,000	400	0	"
6/7/29	25 c.c.				0	"
6/9/29	15 c.c.				0	"

The patient was discharged at the end of eight days, and returned to work. His physician told me three years later that the patient had remained well, and that he continued to drink as much "hooch" as ever.

CASE 3.—Mr. D. W., white, theater organist, aged forty-seven, was seen at the request of Dr. Haller, at the patient's home June 25, 1925.

Present Illness.—Returning home from work about 11:00 P.M., the patient was taken with a sudden sharp severe pain in the head. He remembered nothing after that until he was found in collapse on the stairway of his home. Later, he had an indistinct recollection of crawling home on his hands and knees.

Examination.—The patient looked very ill. He was irrational, stuporous, cyanotic, complaining of severe headache. His neck was stiff, and head retracted. A lumbar puncture yielded 40 c.c. of uniformly bloody fluid under increased pressure. He soon became more rational, and was taken to St. Luke's Hospital. His temperature was 99.4, pulse 66, and respirations 22. The pupils were dilated, equal, regular, and reacted to light and distance. The deep tendon reflexes were normal; there was slight but distinct general rigidity of the body. He complained bitterly of headache, was restless, very active and noisy at times, and cyanotic. There was improvement in his condition following repeated lumbar punctures. When he became mentally clear, he could recall no cranial injury. He was a total abstainer from alcoholics.

Blood.—Erythrocytes 4,800,000; leucocytes 10,600; hemoglobin 80. Wassermann, negative. Coagulation and bleeding time four min. Urine: trace of albumin. No sugar or acetone bodies.

Spinal Fluid.—Wassermann, negative.

SPINAL FLUID

DATE	AMOUNT	PRESSURE MILLIMETERS OF MERCURY	ERYTHROCYTES	LEUCOCYTES	CLOT	SUPERNATANT FLUID
6/25/25	40 c.c.	++	+++		0	Water color
6/26/25	25 c.c.	++	++		0	" "
6/30/25	65 c.c.	26	210,000		0	Amber
7/ 1/25	30 c.c.	++	++		0	"

July 10, two weeks after admission, he was discharged, recovered. Within a week after he left the hospital, he developed lobar pneumonia and died on the fifth day, but without any apparent return of head symptoms.

CASE 4.—Mrs. H., white, housewife, aged sixty-two. Was seen at her home July 8, 1924.

Present Illness.—While sewing, the patient felt a sudden sharp pain in her head, which she described as a blinding pain. She soon became restless, appeared to be in great pain, but did not lose consciousness. Morphine ¼ gr. hypodermically had no effect. Lumbar puncture gave about 40 c.c. of uniformly bloody fluid under increased pressure. The patient was somewhat relieved within two hours. The puncture was repeated twice thereafter, with improvement in and relief of symptoms. She remained at her home, and seemed to have fully recovered in ten days. I have no record of further examinations in this case.

CASE 5.—Mr. A. B., white, laundry wagon driver, aged forty-two, seen with Dr. A. H. Arp, at the Moline Public Hospital, November 16, 1926.

Present Illness.—The patient, apparently well, drove his truck away from the laundry at 2:00 P.M. He was found lying on the seat of the truck about an hour later. The truck had run over the curb into a vacant lot. He remembered having had a severe pain in his head, and that he soon became helpless. He recalled nothing more until the next day.

Examination.—At 6:00 P.M., Nov. 16, 1926, the patient looked very ill. He was comatose; his breathing labored; the skin dry and cyanotic. There was a general rigidity of the body. He tossed from side to side and moaned. His pupils were dilated, equal, round, and reacted to light. The sclerae were deeply injected. Examination of the reflexes was unsatisfactory on account of the rigidity.

The Wassermann both on blood and spinal fluid was negative and the blood pressure was 160/95.

SPINAL FLUID

DATE	AMOUNT	PRESSURE MILLIMETERS OF MERCURY	ERYTHROCYTES	LEUCOCYTES	CLOT	SUPERNATANT FLUID
11/16/26	62 c.c.	+++	+++		0	Water color
11/17/26	40 c.c.	24	+++		0	Straw "
11/17/26	36 c.c.	20	270,000	1,000	0	Amber "
11/18/26	30 c.c.	22	200,000	780	0	" "
11/19/26	30 c.c.	17	220,000	800	0	" "
11/20/26	38 c.c.	10	150,000	1,200	0	" "
11/21/26	20 c.c.	16	80,000	1,300	0	" "
11/22/26	25 c.c.	+	+			

The patient left the hospital at the end of sixteen days, and returned to work two weeks later. His physician reported that he is well now after two and one-half years.

CASE 6.—M., white, practical nurse, aged thirty-four, was seen with Dr. A. E. Williams at the Moline Public Hospital.

Present Illness.—The patient was left in charge of the children in a family while their mother was away from home for the afternoon. The patient seemed to be in her usual good health. When the mother of the children returned, she found the patient lying on a bed unconscious. The first physician who saw her thought she had an epileptic seizure. Dr. W. saw her about an hour later, and sent her to the hospital. She did not regain consciousness. During the evening and forepart of the night, she was quite active at times, and seemed to be in great pain. Toward morning she became deeply comatose.

Examination.—At 1:00 P.M. the next day, or twenty to twenty-two hours after attack, the patient was profoundly comatose. The face and hands were very cyanotic. It seemed that the patient was moribund, and further examination was not attempted.

A lumbar puncture yielded about 50 c.c. of uniformly bloody fluid under greatly increased pressure. It was doubtful whether any improvement followed. She expired about five hours later, as we were preparing to repeat the puncture. I had the feeling at the time, that earlier spinal drainage might have changed the course of events.

CASE 7.—Mr. P. A., white, truck driver, aged sixty. The history and clinical findings are as given by Dr. L. J. Porstmann, Davenport, Ia.

Present Illness.—On February 19, 1929, the patient while at work, suddenly developed a severe headache, became dizzy and ill. There was no evidence then (nor at autopsy later) of any cranial injury. Dr. Porstmann saw him first on the following day. He complained of headache and dizziness, which was not relieved by morphine. Later that day, the patient became irrational, followed by convulsive seizures and loss of consciousness. During the next three days, he became very active and had to be restrained. He was given large doses of morphine. A noncooperative spirit on the part of the family prevented anything more than casual observation in this case. Counsel was not wanted, and permission for a lumbar puncture positively refused. The patient expired February 24, on the sixth day of his illness. At the request of an insurance company, I performed an autopsy about four hours after death, before embalming had been done.

The following brief abstract of the autopsy record gives only the important findings.

There was no sign of trauma or external violence. The scalp and cranium showed no sign of injury. The skull cap averaged from 5 mm. to 11 mm. thick. The dura was everywhere intact, smooth, moist, and normally adherent to the skull. In removing the cap, the arachnoid was inadvertently cut, followed by gush of bloody serous fluid (about 100 c.c. from the saw cut. There was still as much as 75 c.c. of bloody fluid between the arachnoid and pia when the cap had been completely removed. The dural sinuses contained the usual dark red, firm, nonadherent partially clotted blood.

The appearance of the arachnoid was striking. It contained irregular masses of soft blood clot varying in color from light to dark red and from 3 mm. to 10 mm. in thickness. Several stages of organization could be easily distinguished grossly, from strings and plaques of fibrin to collections of semiliquid blood. No single source of the blood could be found. A careful search was made for vascular dilatations, aneurisms in and around the circle of Willis. The most of the blood, and the thicker more organized clots were over the upper half of the cerebrum, and about equally distributed in both hemispheres, possibly a little larger volume on the left side. The ventricles contained a bloody fluid with no sign of clotting. They were of normal size, and the communications between them were patent.

The pia mater was intact, and somewhat edematous. The brain itself was of normal consisteney and contour. There were no gross changes. Serial sections showed no evidence grossly of hemorrhage, thrombosis, embolism or tissue change anywhere in the cerebrum, cerebellum, pons, medulla pedunceles, or upper core. There was no evidence of heart, kidney, pulmonary, or liver disease.

Brief Summary of Seven Cases Reported in the Text of this Paper.

INCIDENCE

CASE	SEX	AGES	OCCUPATIONS
1	Female	12	Schoolgirl
2	Male	49	Carpenter
3	Male	47	Musician
4	Female	62	Housewife
5	Male	38	Delivery man
6	Female	34	Practicel nurse
7	Male	60	Delivery man

CASE	DURATION (DAYS)	OUTCOME	REMARKS
1	5 –14	Recovery	
2	5 –10 and 5 – 8	Recovery	2 attacks
3	7 –14	Recovery	Died of pneumonia
4	4 – 8	Recovery	
5	16 –30	Recovery	
6	1.5–	Death	Delayed drainage
7	5 –	Death	No drainage

ONSET OF SYMPTOMS

1. Engaged in usual occupations, previously well.

2. No history of cranial trauma in these cases.

3. A sudden, sharp, severe pain in the head, soon followed by symptoms of meningeal irritation. Pain usually described as excruciating.

4. Active delirium and partial loss of consciousness.

COURSE OF DISEASE (1)

1. Mental picture dominates with the expression of great pain, restlessness and cerebral impairment varying from delirium to profound coma.

2. Temperatures vary from 97° to 101° F., the pulse 60 to 90, and respirations at first not much altered became slower and more labored.

3. Eyes: pupils dilated, equal, regular, react to light and accommodation. Spontaneous lateral nystagmus present in one case.

4. A definite but not marked general rigidity of the body and extremities. No paralysis.

5. Superficial and deep reflexes not reliably reported in this series. As a group, generally diminished and influenced by mental state.

LABORATORY FINDINGS

1. Blood counts: (a) Erythrocytes, within normal limits. Leucocytes, 10,000 to 14,200. Hemoglobin, 70 to 92. (b) No records of differential counts.

2. Coagulation and bleeding time within normal limits.

3. Wassermann tests all negative in 6 cases, seventh not done.

4. Urine examinations: Usually a trace of albumin. No sugar or acetone bodies.

CEREBROSPINAL FLUIDS

1. Uniformly bloody fluids under increased pressure.

2. Blood content and pressure diminish with successive punctures.

3. Leucocytes slightly increased in proportion to reds.

4. On standing cells settle down to make up as much as 1/20 the volume of the mixture.

5. Early in the attack, supernatant fluid is water color. In successive withdrawals, xanthachromia increases as red cells diminish and show degeneration.

6. There is no tendency to clotting of the fluid.

CONCLUSIONS

1. Seven cases of spontaneous meningeal hemorrhage are reported with reference particularly to the cerebrospinal fluid.

2. In five cases where drainage of the subarachnoid fluid (lumbar puncture) was instituted early and repeated, there was a prompt improvement and recovery. In two cases where drainage was delayed death followed within twenty-four and thirty-six hours respectively.

3. A bloody, nonclotting cerebrospinal fluid under pressure is of great diagnostic importance.

4. As a cause of sudden and unexplained coma, the possibility of spontaneous meningeal hemorrhage should not be overlooked.

5. Ascertaining the cause of coma, particularly in emergencies, has become more and more of a laboratory problem, and in some localities a member of the hospital laboratory personnel is frequently called on to direct the procedure. In fact, it is in recognition of this tendency that one has presented at this meeting what might seem to be more strictly a clinical problem.

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DISCUSSION

Dr. A. S. Giordano.—I would like to add three cases to Dr. Lamb's collection. It is interesting to me the ages in these cases; a boy twelve years of age and a man forty. I performed an autopsy on one out of the three and the other is only a month since the first attack. A man of thirty years of age gradually felt himself going; stopped his automobile and lost consciousness; was brought to the hospital and taken home the following day with a terrific headache. Spinal punctures were done. The fluid was under pressure, bloody and the icterus index was six on the supernating fluid. He was kept in bed for two weeks and gradually improved. Examination revealed a definitely stiff neck. The diagnosis was meningitis partly due to the fact that they had been having considerable meningitis. The blood in the spinal fluid clinched the diagnosis. We gave him rest in bed, transfusions and injections of glucose and he was getting on very well. The nurse left him and they found him unconscious again, with similar findings in the spinal fluid. The family had another physician come in who concurred in the diagnosis. He gradually improved. A boy twelve years of age was playing ball. He stopped playing saying his head hurt him. After thirty minutes he became unconscious and came into the hospital that night. A lumbar puncture revealed tremendous hemorrhage; no clotting observed. He died about two hours after the puncture. Autopsy revealed diffuse subdural hemorrhage involving the entire brain. We could not ascertain the point of bleeding.

Dr. Frederick H. Lamb.—With regard to Dr. Giordano's question, the highest temperature observed in our series of cases was 101° F. It was mentioned that the autopsy findings were subdural hemorrhages. Does this mean literally subdural or subarachnoid?

Dr. A. S. Giordano.—Both.

Dr. Frederick H. Lamb (closing).—My idea of it is this: The subdural hemorrhage or hematoma is the chronic hemorrhagic pachymeningitis of Virchow, usually a delayed manifestation or sequel of cranial trauma; whereas, spontaneous subarachnoid hemorrhage is acute, usually nontraumatic and probably due to the rupture of a small aneurism. The subdural hematoma is amenable to surgical treatment, whereas the spontaneous subarachnoid hemorrhage is not. Frequently, repeated lumber punctures with relief of increased intracranial pressure, not only establish the diagnosis firmly, but give prompt respite of symptoms.

A STUDY ON STREPTOCOCCI IN RELATION TO PATHOGENICITY AND SUGAR FERMENTING PROPERTIES*

A Pathologic Classification of Streptococci

By L. G. Hadjopoulos, M.D., and Reginald Burbank, M.D., New York City, N. Y.

OF LATE years, the important fact that streptococci do not comprise a homogeneous group has been steadily gaining credence.[1] When first isolated from septic wounds, streptococci were thought to play a simple pathogenic rôle; but, from the further advance of bacteriology, they have been isolated from various sources, pathogenic as well as nonpathogenic; so that even now, in consideration of their rôle as secondary invaders, knowledge of their specific pathogenicity is very confusing.

The study of streptococci really started with improvement of technic in blood culture methods, which resulted in the recognition of certain types of true streptococcic septicemias, such as puerperal fever, malignant endocarditis, septicemias from focal infections, etc. An opportunity was thus afforded of observing certain important biologic characteristics not common to all types, and the heterogeneity of the streptococcic group became apparent. Since then, methods have been further improved, and new ones devised that have permitted more scientific study of their biologic differences. Certain specific types, such as the Streptococcus hemolyticus, the Streptococcus pyogenes, and the Streptococcus viridans "mitis" of endocarditis, the weak hemolytic streptococcus of scarlatina, and the Streptococcus erysipelatis have been differentiated. There is no doubt that persevering research will finally classify streptococci, not merely according to their morphology, cultural characteristics, and intricate biologic properties (factors which are not always constant), but also according to that which is the ultimate aim of bacteriology; namely, their specific pathogenicity.

In the course of the last three years we have collected a series of cases of streptococcic septicemia, and by applying recent biologic classifications have compiled Table I. The nomenclature used by Holman,[2] based on the fermentation of his three cardinal sugars, was used throughout.

General Remarks: The clinical classification of certain infectious diseases into acute and chronic types has a bacteriologic parallel in the blood altering properties of the streptococci that cause them. The acute conditions are caused usually by hemolytic streptococcus; the chronic, by Streptococcus viridans and by nonhemolytic streptococci.

In eleven cases of mastoiditis hemolytic streptococci were found in ten of them and Streptococcus viridans of the alpha prime type, also weakly hemo-

*From the Pathological Laboratories of Beth Israel Hospital, New York, and Laboratory, 6 East Seventy-eighth Street, New York.

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lytic, was found in the other case. Four cases of acute septicemia following infected wounds were all hemolytic. Streptococcus viridans were found in ten patients with subacute bacterial endocarditis. The occurrence of both streptococcus hemolyticus and Streptococcus viridans in multiple infectious arthritis speaks also in favor of the peculiar course of this disease as regards chronicity.

TABLE I

A PATHOLOGIC CLASSIFICATION OF STREPTOCOCCI OF SEPTICEMIC ORIGIN

PATHOLOGIC CONDITION	HEMOLYTICUS					VIRIDANS				
	INFREQUENS	PYOGENES	ANGINOSUS	EQUI	SUBACIDUS	FECALIS	MITIS	SALIVARIUS	IGNAVUS	TOTAL
Rheumatoid Arthritis	8	1	2		1	12	7	3	2	36
Bacterial Endocarditis							3	5	2	10
Acute Mastoiditis		6	3	1			1			11
Acute Rheumatic Fever		1					1			2
Acute Septicemia			4							4
Total	8	8	9	1	1	12	12	8	4	63

MULTIPLE INFECTIOUS ARTHRITIS

As we have dealt with this subject in previous contributions[3, 4] and intend to deal with it more extensively in a subsequent work, we shall merely mention here certain facts of importance. These positive blood findings were obtained mostly in afebrile cases by means of a special technic depending on the inactivation of the bacteriolytic complement in the blood before it was cultured. Streptococcus infrequens under hemolyticus, and Streptococcus fecalis under viridans were the most frequently isolated. Biologically the difference between Streptococcus infrequens and fecalis is based on hemolysis only. Their sugar fermentations are identical. The isolation of a fair percentage of other streptococci from the blood of arthritic patients still lacks an adequate explanation; for, in our experience, with the exception of Streptococcus fecalis and infrequens which never failed to produce arthritis in rabbits, the only other streptococcus that occasionally produced arthritis was the mitis. Streptococcus anginosus and Streptococcus pyogenes were too virulent to cause a chronic disease. Experimentally, they invariably gave rise to an acute fatal septic condition, and, occasionally, a pyogenic joint in the case of Streptococcus pyogenes. We were never successful in producing arthritis with Streptococcus salivarius, subacidus, and ignavus. Their presence, therefore, in the blood of arthritic patients in all probability has no important bearing on the disease.

ACUTE RHEUMATIC FEVER

Our list in this category is very meager. In fact, we had only one true case whose blood culture gave Streptococcus mitis, raffinose positive and inulin negative. The other case with the Streptococcus pyogenes finding proved to be a pyogenic, post-traumatic knee-joint infection, confirmed also by the post-operative findings.

TABLE II

A CLINICAL AND BACTERIOLOGIC ANALYSIS OF TEN CASES OF BACTERIAL ENDOCARDITIS

BACTERIOLOGIC DATA								CLINICAL DATA														
				SUGAR REACTION				1	2	3	4	5	6	7	8	9	10	11	12	13		
SERIAL NUMBER	NAME	TYPE OF ORGANISM	NUMBER OF COLONIES PER PLATE	MANNITE	SALICIN	LACTOSE	HOLMAN CLASSIFICATION	RHEUMATIC HISTORY	SIGNS OF FOCAL INFECTION	PAIN (EXTRA-CARDIAC)	PETECHIAE: MUCOUS MEMBRANE	PETECHIAE: SKIN	FEVER: SUSTAINED	FEVER: SEPTIC	LEUCOCYTE COUNT	SEGMENTED FORMS	CARDIAC PATHOLOGY	ELECTROCARDIOGRAPHIC RECORD: P	ELECTROCARDIOGRAPHIC RECORD: QRS	ELECTROCARDIOGRAPHIC RECORD: T	ELECTROCARDIOGRAPHIC RECORD: DESCRIPTION	OUTCOME
1	F.	St. Vir.	100	–	–	+	Salivarius	+	+	+	+	+	–	+	9	76	M	–	+	+	Inverted T, LVP	Died
7	H.	" "	100	–	–	+	"	+	+	+	–	–	–	+	—	—	AM	–	+	+	Inverted T, LVP	"
10	W.	" "	15	–	–	+	"	+	+	+	+	+	–	+	13	81	TAM	–	+	+	Inverted T, LVP	"
29	M.	" "	50	–	–	+	"	+	+	+	–	+	–	+	11	84	M	–	+	+	Inverted T, LVP	"
30	S.	" "	100	–	–	+	"	+	+	+	–	+	–	+	—	—	M	—	—	—	No record	"
5	L.	" "	5	–	–	–	Ignavus	–	–	–	+	–	+	–	47	78	M	+	–	–	Peaked Wide A	"
11	W.	" "	100	–	–	–	"	–	–	–	–	–	+	–	21	65	M	+	–	–	Peaked Wide A	Recovered
9	E.	" "	30	–	+	+	Mitis	–	+	–	+	–	+	–	10	80	M	+	–	–	Auric. Hypert.	Died
6	R.	" "	40	–	+	+	"	Historical data missing														"
3	L.	" "	100	–	+	+	"	Historical data missing														"

NOTE.—The plus sign indicates the presence of the particular clinical condition. Under sugar reactions it indicates fermentation.
The leucocyte count gives the figures in thousands.
In Column 10, M indicates mitral valvular, AM mitral and aortic, and TAM tricuspid mitral and aortic.
LVP stands for left ventricular preponderance and A for auricular hypertrophy.

BACTERIAL ENDOCARDITIS

Comments: In ten cases of typical bacterial endocarditis we found three different types of streptococci. We are thus confronted with the dilemma of either giving up the attempt at a pathologic classification of streptococci based on certain biologic characteristics, or of demonstrating that all bacterial endocarditides are not the same.

To accept the latter hypothesis as correct, it is not sufficient to show the existence of certain symptomatic variations in the clinical course of individual endocarditides, but it is necessary to prove that such variations occur repeatedly in a proportion of cases sufficient to constitute a group. Furthermore, given that the symptomatology of endocarditis warrants such a grouping, we still have to find whether this has a bearing on the specific etiologic factor.

We entered very carefully into an analytic study of the symptomatology and clinical data of the above ten cases in an attempt to prove to our own satisfaction whether or not our arbitrary biologic classification of the above streptococci had a clinical foundation. Omitting symptoms and signs common to all three groups, we recorded in Table II such data as were conspicuously dissimilar.

The differences were classed under the following six heads:

Columns 1, 2, 3: Previous rheumatic history, positive focal findings, rheumatic pains (other than directly attributable to the cardiac condition).

Columns 4, 5: Occurrence of petechiae in mucous membranes and skin.

Columns 6, 7: Type of fever (sustained, septic).

Columns 8, 9: Leucocyte count and differential.

Column 10: Cardiac signs by physical examination.

Columns 11, 12, 13: Electrocardiographic evidences of the extent of cardiac involvement.

In the bacteriologic group of Streptococcus salivarius we note the existence of a rheumatic history; the persistence of active infected foci and joint pains; the generalized distribution of petechiae both in mucous membranes and extensively in the skin; the septic type of temperature, at times fluctuating between 104° F. and 98° F.; the relative leucopenia (the table gives the average of 10 or 15 counts in each case), with a high percentage of segmented forms: the complex endocardial involvement, double mitral in Cases 1, 29 and 30, double mitral and aortic in Case 7, and double mitral, aortic, and tricuspid involvement with partial bundle block, right, in Case 10. The electrocardiograms disclosed in addition to the (left) ventricular preponderance, myocardial involvement as evidenced in the inversion of the T-wave in all, and notching of the S in Cases 10 and 29. No signs of auricular abnormalities were detected.

In contrast to the group of Streptococcus salivarius which proved to be very rich in symptomatology, we take the Streptococcus ignavus group where the absence of most of the above symptoms is conspicuous, viz.:

The absence of rheumatic history and its concomitant signs.

The absence of petechiae in skin and mucous membranes with the exception of a suspicious spot in the conjunctiva of Case 5.

The sustained course of temperature unlike the septic fever of Streptococcus salivarius.

The marked leucocytosis with a low percentage of segmented forms, a marked contrast to the hematology of Streptococcus salivarius.

The involvement of the mitral valve alone.

The sparing of myocardium with an auricular hypertrophy, as evidenced in the P-waves of both cases.

We cannot ignore such a marked contrast irrespective of the small number of cases that comprise our groups. Furthermore, the unfailing coincidence of the characteristic group symptoms under our attempted bacteriologic classification shows the importance of such concept.

The group of Streptococcus mitis takes an intermediate position between the Streptococcus salivarius and Streptococcus ignavus:

A rheumatic history and active focal signs may or may not be found.

Petechiae are limited to the mucous membranes (conjunctiva in Case 9).

The type of fever is rather of the sustained type.

The leucocyte count is around normal, with a marked polynucleosis.

The mitral valves are usually involved and the myocardium may undergo pathologic changes.

It is unfortunate that our findings could not also be substantiated by necropsy records.

MASTOIDITIS

In our group of eleven cases of mastoiditis Streptococcus pyogenes occurred seven times, Streptococcus anginosus twice, and Streptococcus mitis and equi each once. Here, again, we meet the same difficulty of accounting for four types of streptococci in the same disease. By a careful analytical study of the clinical data we were able, nevertheless, to differentiate at least two different types; the pyogenes and the nonpyogenes. The data are given in Table III.

Comments: The eleven cases of mastoiditis in respect to their bacteriologic and clinical features fall into two distinct categories:

First, the Streptococcus pyogenes was characterized by metastatic abscesses in remote areas, the site of which determined the final outcome of the particular case. When the brain or meninges was involved, it proved 100 per cent fatal (Cases 4, 13, 28). Recovery, however, was the general rule with soft tissue involvement, as such locations were usually accessible to surgery (Cases 14, 27). Occasionally, the course of the disease was so severe and toxic that death supervened in a short time, thereby offering little chance for a metastatic abscess (Case 22).

Second, all other streptococcic groups combined, which were characterized by the absence of pyogenic metastases, except complications occurring in the immediate neighborhood of the mastoid, such as sinus and perisinus involvement. It was significant to note that there was no fatality in this group.

Neither the severity of symptoms at the onset and during the subsequent course of the disease, nor the type of fever were pathognomonic of the kind of streptococcus isolated. Symptoms, as such, were equally partaken of by all

TABLE III

A CLINICAL AND BACTERIOLOGIC ANALYSIS OF ELEVEN CASES OF ACUTE MASTOIDITIS ACCOMPANIED BY SEPTICEMIA

		LABORATORY DATA						CLINICAL DATA				
				SUGARS FERMENTED								
SERIAL NUMBER	NAME	TYPE OF ORGANISM	NUMBER OF COLONIES	MANNITE	SALICIN	LACTOSE	HOLMAN CLASSIFICATION	LEUCOCYTE COUNT	SEGMENTED FORMS, %	POSTOPERATIVE DIAGNOSIS	COMPLICATIONS	OUTCOME
4	P.	Streptococcus Hemolyticus	50	−	+	+	Pyogenes	22	91	Acute suppurative sclerosed	Brain abscess	Died
13	P.	" "		−	+	+	"	26	73	Operated outside	Meningitis	Died
14	K.	" "		−	+	+	"	16	71	Acute suppurative granulating	Subdural abscess	Recovery
25	A.	" "	50	−	+	+	"	19	84	Chronic purulent necrotic	Gluteal abscess	Died
22	R.	" "	18	−	+	+	"	—	—	Acute fulminating	Died in 2 days	Died
27	S.	" "	50	−	+	+	"	11	80	Acute hemorrhagic necrotic	Femoral abscess	Recovery
28	A.	" "		−	+	+	"	15	70	Chronic purulent necrotic	Meningitis	Died
12	B.	" "	5	−	−	+	Anginosus	10	78	Recovery without operation	None	Recovery
24	A.	" "	25	−	−	+	"	11	80	Chronic sclerotic granulating	Sinus phlebitis	Recovery
17	G.	" "	3	−	+	−	Equi	8	60	Acute suppurative	Sinus thrombosis	Recovery
8	B.	Streptococcus Viridans	30	−	+	+	Mitis	8	70	Acute hemorrhagic necrotic	None	Recovery

Note.—The leucocyte count gives the figures in thousands.

TABLE IV

A CLINICAL AND BACTERIOLOGIC ANALYSIS OF FOUR CASES OF ACUTE SEPTICEMIA WITH NO TENDENCY TO LOCALIZATION

SERIAL NUMBER	NAME	LABORATORY DATA							CLINICAL DATA				
		TYPE OF ORGANISM		NUMBER OF COLONIES	SUGARS FERMENTED			HOLMAN CLASSIFICATION	LEUCOCYTE COUNT	SEGMENTED FORMS, %	CLINICAL DIAGNOSIS	PRIMARY SOURCE OF INFECTION	OUTCOME
					MANNITE	SALICIN	LACTOSE						
26	W.	Streptococcus	Hemolyticus	3				Anginosus	11	75	Puerperal sepsis	Unknown	Recovered
21	S.	"	"	50	−	−	+	"	10	90	Chronic cardiac arteriosclerosis	Phlebitis	Died
19	M.	"	"	3	−	−	+	"	12	85	Erysipelas	Paronychia	Died
16	S.	"	"	3	−	−	+	"	13	80	Gangrene of finger	Operative infection	Recovered

streptococci. Severe septic fevers, though common with Streptococcus pyogenes, were not at all uncommon with such other streptococci as occur in mastoiditis. Of clinical data the hematologic picture was all that showed certain group characteristics. In the Streptococcus pyogenes group there was a marked leucocytosis, with a relative increase of the segmented forms; while the nonpyogenes group gave either a normal count or a relative leucopenia (Cases 8, 12, 17, 24.)

Therefore, depending on the leucocyte count alone, we venture to make the following statement relative to prognosticating the outcome of a mastoid condition:

1. If the leucocyte count is above 10,000, the involvement is probably due to Streptococcus pyogenes, carrying a high percentage of mortality, the latter depending on the particular localization of the metastatic abscess.

2. If around 10,000, it is usually caused by Streptococcus anginosus, and

3. If below 10,000 Streptococcus equi or Streptococcus viridans mitis; both latter groups carrying a very low mortality.

UNCLASSIFIED SEPTICEMIAS

This group of acute infections is generally called cryptogenic septicemia because the activity of the original focus being not conspicuous or having ceased to be active, leaves small clue to its origin. By a careful study of the history, however, one can often trace the source. It is usually a minor accidental infection of the extremities (Cases 16, 19, and probably 21), or of the mucous membranes, especially the nasal accessory sinuses. In Case 26 the genital tract was suspected.

In view of the acute course of the disease in this group it was natural to suspect a hemolytic microorganism as the cause. We were impressed by the detection in the blood of these cases of a single type of streptococcus, the anginosus, with only slight variation in its raffinose fermenting property.

As stated above, we have detected this same type of microorganism in a small percentage of cases of acute catarrhal mastoiditis. If, then, Streptococcus anginosus had a specific selectivity for the mastoid, the absence of mastoid symptoms in the cases under discussion could be explained only by the remoteness of atria to the mastoid and the relative shortness of the disease, although we had two cases with recovery where such a localization would naturally be expected. Judging from the blood reaction, we presume that in both instances we were dealing with the same type of microorganism. The leucocyte count in mastoids caused by Streptococcus anginosus varied between 10,000 and 11,000 with a differential of 80 per cent segmented and 20 per cent monocytes. The average count in the nonmastoid septicemias was 11,500, with a differential of 85 per cent.

CONCLUSIONS

The differentiation of streptococci into hemolytic and viridans is of sufficient clinical value to enable us to divide infectious conditions into acute (usually caused by the hemolytic) and the subacute or chronic (caused by the viridans).

By the introduction of their sugar fermenting characteristics we take a further step in their pathologic classification.

In addition to the already known specific types (Streptococcus scarlatinae, Streptococcus erysipelatis) we feel justified in also differentiating the following:

Streptococcus infrequens, and Streptococcus fecalis in subacute and chronic rheumatoid arthritides.

Streptococcus salivarius, mitis and ignavus in subacute and chronic bacterial endocarditides.

Streptococcus pyogenes, anginosus, and equi in acute mastoiditis.

Streptococcus anginosus in cryptogenetic (accidental) septicemias.

As all streptococci partake to some degree of the general group characteristics, we would naturally expect some overriding in the symptomatology of the class of infections to which they give rise. Nevertheless, depending on their individual characteristics, they further subdivide known pathologic conditions into clinical subgroups. We thus have a salivarius endocarditis with myocardial as well as endocardial involvement, extensive petechial rash on the skin and mucous membranes, and a relatively normal white cell count and differential. In contrast to the salivarius we have another type, the ignavus endocarditis running a less severe course, not involving the myocardium, with no petechiae on the skin, and with a markedly high leucocytosis. In the case of mastoiditis we distinguish a pyogenic form apparently invariably caused by Streptococcus pyogenes and characterized by suppuration of the mastoid cells with metastatic abscesses either in the brain, meninges, or the soft tissues, coupled with a high leucocytosis. Mortality in this group is very high. The other types (anginosus, equi, and mitis) are not strictly pyogenic (with the possible exception of equi), but give rise to an acute catarrhal, hemorrhagic, or necrotic inflammation with absence of metastatic abscesses and a relatively low leucocyte count.

Although the above generalizations should be taken reservedly, due to the small number of cases observed, still we consider them worth mentioning as the possible start of a working basis for the ultimate pathologic classification of streptococci.

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6 East Seventy-Eighth Street.

A DETERMINATION OF THE EFFECTIVENESS OF ACIDOPHILUS CULTURE IN FUNCTIONAL DISTURBANCES OF THE COLON*

Clinical and Laboratory Results in a Series of Dispensary Patients

By Lewis H. Hitzrot, M.D.,† and Frank B. Lynch, Jr., M.D.,‡
Philadelphia, Pa.

THIS study extends an investigation reported by Lynch[1] in 1928, in which a comparison was made of the implantability of the X and Y strains of Lactobacillus acidophilus in a series of institutionalized epileptics. The present observations were carried out in a group of unselected gastrointestinal out-patients. Such a group partakes of many of the features of an office practice, and it was hoped that the findings would give some indication of what may be expected under conditions of only partial control of the patient's habits and diet. For despite the numerous excellent laboratory and clinical studies to be found in the literature, led by those of Cheplin and Rettger[2, 3] the application of the advocated therapeutic methods to routine practice demands further study.

The historic data of the problem of influencing for good the bacterial content of the bowel are so completely summarized in the monograph of Rettger and Cheplin that they will not be repeated. A complete bibliography to 1921 is given by those authors and is brought to 1927 by Cheplin[3] and Cruickshank.[4] The recent contribution of Sagastume and Solari[5] gives evidence that the acid gastric juice destroys the L. bulgaricus, probably accounting for Rettger and Cheplin's finding, confirmed by others, that the bulgaricus organism cannot be implanted. On the other hand, L. acidolphilus was not appreciably affected, according to the investigators. No further reports on acidophilus therapy were found in the literature of the past eighteen months.

Granting that L. acidophilus can be made to supplant the normal proteolytic group of intestinal organisms, and granting that this is of great clinical benefit in many refractive cases of constipation, colitis, or "indigestion," as previous studies have indicated, there remains the question of the most practicable method of administration. Large daily quantities of viable organisms, reinforced with enormous amounts of lactose or dextrin, have often been proved to accomplish implantation.

The present study comprises observations on two series of patients. Our object, in the first series, was to determine, if possible, whether a nominal number of viable organisms administered in broth culture rather than milk,

*From the Gastro-Intestinal Clinic of the Hospital and the William Pepper Laboratory of Clinical Medicine of the University of Pennsylvania.

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†Instructor in Medicine, Assistant Physician, Medical Division, Hospital of the University of Pennsylvania.

‡Dickson Fellow and Associate in Bacteriology, William Pepper Laboratory of Clinical Medicine, University of Pennsylvania.

over several weeks, either alone, or with small amounts of lactose, could bring about a modification of the fecal flora with alleviation of symptoms.

In the second series of patients the X and Y strains were compared for implantability and clinical effect.

METHOD

The first group of 18 patients, 15 of whom continued until the studies were completed, was used to compare the results obtained by feeding moderate daily amounts of lactose alone, of broth culture of Y acidophilus organisms alone (50-75 billion organisms), and of combined lactose and culture, each over a period of eight weeks. Four members of the group were used as controls, and received no medication; 4 received the lactose; 3 the culture, and 4 the culture reinforced with lactose. The diagnoses, determined beforehand in the routine workup of the clinic, included constipation, chronic colitis, cholecystitis, pylorospasm, and marginal ulcer after gastroenterostomy. Selection was made only on the basis of willingness and ability to cooperate throughout the extended period. The possible therapeutic results were explained to the patients individually, their promise to cooperate secured, and all medication, except laxatives as necessary, stopped.

The technic of study of the specimens was similar to that described by Lynch,[1] modified in minor details. Each patient was observed for three weeks, reporting his daily habits and symptoms, and type of stool. Likewise three weekly stool specimens were examined grossly, by Gram stain and by culture. Thus, with the patient's tabulated report, a level of habits and of the normal proportions of bacterial flora was established.

At the end of three weeks, administration of culture was begun in 11 of the 15 patients of the group, the remainder acting as controls. Each patient was given weekly a supply of lactose, fresh broth culture, or both culture and lactose, sufficient to last until his next visit. He received also a container for the fresh stool specimen, and a mimeographed card on which to keep daily record of the number and type of stools, laxatives, or enemas, change in symptoms, etc. The latter were checked and noted from time to time by one of us as the patient returned for his week's supplies. It is realized that the inability to control the patient in his home opens the clinical interpretation of his report to error and inaccuracy; and it can merely be stated that there was every reason to believe that the cooperative patients who completed the course followed instructions consistently.

The weekly specimens were noted as to color, consistency, age, and odor. A small portion of the mixed specimen was then emulsified in sterile distilled water by an electric stirring machine. A portion of the emulsion next was diluted to an opacity of 10-12 on the McFarland nephelometer scale[6] and two pour-plates of casein-digest agar inoculated with 1 millionth and 1 ten-millionth of a cubic centimeter of the emulsion respectively. The plates were examined after forty-eight hours' incubation with a No. 10 ocular and 25 mm. objective for acidophilus colonies. Thin films of the original emulsion were gram-stained by the modification of Kopeloff and Beerman,[7] and the proportions of gram-positive and gram-negative bacilli recorded by counting 100 or 200 organisms in various fields.

RESULTS

Bacteriologic Findings.—The gram-stain counts, made weekly, showed in Series 1 a consistent rise in the gram-positive bacilli only among the patients receiving both culture and lactose. The average increase was 10 per cent, over the eight-week period. With culture or lactose alone, as well as with the controls, the fluctuation was insignificant.

Weekly colony-counts on the agar pour-plates revealed a transient implantation in only one patient, the averages for the others ranging from an occasional acidophilus colony to 7 per cent. Two individuals showed no acidophilus colonies in any of the cultured specimens, thereby resembling the controls. Furthermore, our present laboratory results serve to confirm the statement made before that identification of the Y acidophilus colonies on culture plates is extremely difficult, and that the effectiveness of the medium is a most variable factor. The figures obtained, we feel, give inadequate record of the viable acidophilus organisms in the specimen. In short, it is again stated that the present methods of plate culture, so far as the Y strain of L. acidophilus is concerned, are unsatisfactory. The gram-staining, however, was found satisfactory as an indication of alteration from the proteolytic gram-negative group to the acidophilus gram-positive group. The evidence, of course, that such gram-positive organisms are L. acidophilus, is merely presumptive.

In general, the laboratory results were disappointing. The viability of the cultures was assured by control plating; the strains had been isolated from feces so as to assure their implantability in the human intestine. Yet with fairly large dosage, only that group using lactose as fortifying agent showed any appreciable increase in gram-positive organisms.

Clinical Findings.—From a clinical standpoint the results were more encouraging. All constipated patients agreed that the stools were a little softer, less foul, and in some instances, laxatives were less frequently used. One patient, with a long-standing colitis, reported consistent improvement in the character of evacuations. No one was made worse. No patient developed diarrhea from the three tablespoons of lactose. The broth culture was not a disagreeable dose to take, and caused no gastrointestinal symptoms.

SECOND SERIES

The above group received only the Y strain of organisms. A second group of patients was therefore assembled in which a comparison of the implantability of the X and Y strains was attempted. Three tablespoons of lactose daily were prescribed for all of this group except the controls. Here the conditions under which the two cultures were given were made as near alike as possible. The strains were new ones kindly furnished us by Dr. Harry A. Cheplin of the H. K. Mulford Laboratories. Fresh cultures were made each week as before, and a week's supply of culture and lactose was furnished each patient when he presented the specimen for examination with his daily record of defecation, laxatives, etc.

Two controls, and five patients each of the X and Y group began the course. One patient receiving X organisms and one receiving the Y were

forced to discontinue because of intercurrent illness. Four in each classification, however, continued for the five weeks of study. Two preliminary examinations were made before medication was begun. The technic described above was used.

RESULTS

Although the patients resembled those of the previous group in that they formed a cross-section of the clinic, only one failed to show a rise in the average of gram-positive flora. This seemed to confirm the desirability of the fortifying lactose. With the X organism the average increase was 15 per cent; in the Y group, 6 per cent. Again the plate cultures of diluted specimens proved relatively unsatisfactory as an index of viable acidophilus organisms. The results on dextrose whey agar were compared with peptone tomato-juice agar, and the colony counts were equally unsatisfactory. Two members in each group, however, consistently showed appreciable increases in acidophilus colonies.

The results would indicate also that the effectiveness of the Y strain is less apparent. Clinical improvement was slight, and the gram-stain figures less altered. The small number of patients observed, however, precludes any generalization.

Patient C.Z., in the X group, obtained a true implantation. He had been taking milk regularly in his daily diet, because of a fairly recent gastroenterostomy for peptic ulcer. The beneficial effects of large quantities of sweet milk in this type of treatment are obvious, and a further study is contemplated using milk as a staple of diet, in order to obtain more thorough implantation in such ambulant patients.

An impression gained from previous experience in prescribing acidophilus milk, or broth culture, namely, that constipation, flatulence, or mild colitis symptoms may be markedly benefited, without much objective, bacteriologic change was borne out by the improvement described by the patients although implantation was obviously not achieved. The single colitis patient in this group obtained considerable relief, and has continued the régime.

SUMMARY

Two groups of ambulant gastrointestinal clinic patients were studied over eight- and five-week periods respectively to determine:

First, the relative effectiveness of lactose, of broth culture of L. acidophilus, and of the combined culture and lactose in transforming the fecal flora and ameliorating common symptoms of intestinal stasis or irritation.

Second, the relative implantability of the X and Y strains of L. acidophilus.

Observation of the first series indicates that lactose alone in daily dosage of 20 gm., or of the culture alone in dosage of 50 to 75 billion organisms, is in general not sufficient to raise appreciably the proportion of gram-positive fecal organisms. Combining the culture and lactose brought about a partial change in the gram-stain picture.

Clinical improvement was described in several instances, despite insignificant bacteriologic change in the weekly specimen examined.

Analysis of the results in the second series of patients indicates that while there is little difference in the implantability of the X and Y strains, the present figures tend to favor the X organisms, i.e., the particular small group studied showed more tangible clinical benefit and concomitant bacteriologic result.

The configuration of the X colonies in plate culture, furthermore, makes them more easily distinguished from the colon or enterococcus colonies. To determine an accurate count of Y colonies by the present culture methods was found a difficult and unprofitable task, in the absence of massive implantation.

The most striking results were obtained in a patient (C.Z.) who continued a previously imposed partial milk diet. The ease with which the acidophilus organisms became implanted suggested further that in investigation toward the most practicable method of securing implantations in an office or dispensary practice, a study be made of the effect of a partial sweet milk diet. The disadvantages of acidophilus milk, of reinforcing of the agar or broth cultures with large doses of lactose may be overcome by such a régime. It is hoped to extend this phase of the study further.

Finally, it should be recorded that in our experience, dextrose broth affords a palatable and efficient vehicle for supplying viable L. acidophilus in weekly quantities. The culture retains its potency for about ten days if kept in the cold.

CONCLUSIONS

1. Acidophilus culture alone was found in the present study not to be effective in modifying the fecal flora.

2. Lactose alone in an easily tolerated dose was not found effective.

3. The culture should, we feel, be reinforced by daily doses of lactose up to toleration, doses of 20 gm. of the sugar daily having proved relatively ineffective.

4. Close observation of two groups of patients receiving the acidophilus organism, indicated, however, that frequently symptoms originating in the colon may be markedly ameliorated without laboratory evidence of successful implantation.

5. The X and Y strains, being variants of the same acidophilus organism, were again found almost equally useful. The advantage lay with the X organism chiefly because of ease of identification in plate culture. Its colonies were noted, as reported by others, often to revert to the Y type, however.

6. For the above reasons, and because of the complicated technic of culture, we feel that in routine practice the gram-staining of a film of emulsified feces is sufficient to obtain an indication of implantation. This should be done frequently, however, in every case possible, for implantation is not easy or simple of accomplishment. If clinical results are not forthcoming, the need of drastic upward modification of the dosage of sugar or of both sugar and culture may be indicated by the microscopic picture.

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NOTE ON THE SERUM PROTEIN CONCENTRATION IN A CASE OF MULTIPLE MYELOMA OF THE PLASMA CELL TYPE*

By Roger S. Hubbard, Ph.D., and C. E. Case, M.D., Clifton Springs, N. Y.

IN MARCH, 1928, Perlzweig, Delrue and Geschickte[1] reported results of protein studies upon the blood serum of a patient suffering from multiple myeloma of the plasma cell type. Using the macro Kjeldahl technic and the method of Howe,[2] they found a marked increase in the total protein with the increase almost wholly confined to the globulin fraction. Albumin was somewhat low. The clot which formed after the blood was drawn retracted but slightly, and difficulty was found in obtaining enough serum for analysis. They gave a brief review of hyperproteinemia figures in the literature and showed that their findings were hardly approached by any others reported. Further evidence of the unusual nature of their results is shown by the figures given in a more recent article by Starlinger and Winands.[3] Among 481 determinations on 241 patients suffering from many different diseases there was none which showed such a high value for total protein as did this case of Perlzweig's and only one in which a value even approaching his figures was noted. No globulin concentration approximating those results was recorded. In a review of the literature for other determinations in such conditions they found only two papers bearing on the question. Jacobson[4] isolated and weighed the Bence-Jones protein from the serum of a patient with this compound in the urine, and Rowe[5] in a case of "myeloma of the spinal cord" with the same abnormal constituent in the urine found a normal total protein value and normal concentrations of globulin and albumin in blood serum. A further search of the literature has failed to reveal other reports upon similar material.

Recently an opportunity for studying a case of multiple myeloma of the plasma cell type presented itself. The clinical details of the case have been described elsewhere.[6] Diagnosis was made from x-ray findings, from an examination of the type of cells in the circulating blood, from a microscopic study of a section of bone removed at biopsy, and from the repeated demonstration of Bence-Jones protein in the urine. The presence of relatively large amounts of this substance—0.9 volume per cent and 13 gm. in twenty-four hours in one specimen—and the almost complete absence of other forms of urine protein seem to be the only marked differences between this case and the one reported by Perlzweig and his coworkers.

When blood was taken for analysis, there was no unusual behavior of the clot, and serum for analysis was easily obtained. Determinations of protein in this material and in plasma were carried out by the micromodification of Howe's method described by Hawk and Bergeim.[7] The results are given in the table. Those recorded under the date of November 8 are the averages of satisfactory duplicates. Upon the other specimen only a single determination of each fraction was made. The second specimen was obtained five days after the biopsy. The chief difference between the two analyses is a decrease in the total protein concentration brought about mainly by a lowering of the

*From the Clifton Springs Sanitarium and Clinic, Clifton Springs, N. Y.
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globulin fraction. The unusually low globulin value recorded is hard to explain. In the method used this figure is calculated from the difference between actual determinations of the total protein and albumin, and therefore is liable to a rather large error; but not only was this result based upon duplicate determinations, but the plasma and serum analyses checked each other in a satisfactory manner.

The results, as compared with those of Perlzweig and his coworkers, show comparable high values for fibrinogen, normal rather than low values for albumin, and normal to low instead of high figures for total protein and globulin. These last differences are much too large to be attributed to technical errors, or to be explained by differences in the methods used. It seems not unlikely that the excretion of rather large amounts of Bence-Jones protein, rather than its possible retention in the body, may explain the difference between the findings in this case and in that presented by Perlzweig. As already pointed out, the chief difference between the two patients was the ease with which this compound could be isolated in our case as contrasted with the difficulty in demonstrating it in the other. Perlzweig has suggested that the hyperproteinemia reported by him is an expression of a systemic reaction to a foreign protein. Such an explanation would be entirely satisfactory for the difference between the findings, as the reaction of two individuals to the same foreign protein may be entirely different.

Our figures showing normal to low values for protein and globulin in a case of multiple myeloma of the plasma cell type with Bence-Jones protein in the urine are reported to show that the opposite findings cannot be considered specific for and diagnostic of this condition.

TABLE I*

	OCT. 16, 1928		NOV. 8, 1928	
	SERUM	PLASMA	SERUM	PLASMA
Total protein	7.30	8.94	5.27	6.02
Albumin	5.56	—	4.54	4.55
Globulin	1.74	—	0.73	—
Fibrinogen		0.79		0.81

*Results expressed as per cent by volume.

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LABORATORY METHODS

A CLOCK-TIMED GRAVITY DEVICE FOR DELIVERING SOLUTIONS AT CONSTANT RATES INTRAVENOUSLY*

By A. R. Colwell,† M.D., Chicago, Ill.

Woodyatt's[1] well-known apparatus for delivering solutions intravenously at constant rates fulfills its purpose admirably for relatively short periods. Its use in prolonged experiments lasting overnight is hardly feasible, however, because it might be damaged seriously if left running for long periods unattended. The machine described herein obviates this difficulty. In principle it consists of a device which regulates automatically the rate of flow of a solution suspended several feet above the subject.

The essential working parts are represented semidiagrammatically in Fig. 1. The flow is regulated by means of two syringes, with weighted pistons, mounted vertically on a valve housing *(VH)* inside of which a tapered valve *(V)* rotates. The latter is simply a double two-way valve constructed so that every quarter-turn the connections between the syringes and the inlet *(I)* and outlet *(O)* tubes are reversed. The suspended fluid enters the valve housing through the inlet tube and nipple. With the valve in the position shown the fluid fills the syringe (S_2) by raising the piston (P_2) to the top stop *(T)*. After an interval the valve rotates one-quarter turn and the weighted piston, descending by force of gravity, expels the contents of the syringe through the outlet tube *(O)* into the vein. Simultaneously the inlet is connected with and fills the other syringe (S_1), which had been emptied with the valve in the first position.

The valve is rotated and timed by means of the mechanism shown at the right of the diagram. The motive force for the rotation is provided by a weight *(W)* suspended by a string wound on a spool which is fixed to a large gear *(LG)* in mesh with a small gear *(SG)* on the valve shaft *(VS)*. The trip-wheel *(TW)* at the end of the valve shaft is provided with four teeth mounted alternately at its periphery in the planes of the valve holes. In juxtaposition and at right angles with this wheel is the gear *(G)* which is mounted on the minute-hand shaft *(MS)* of an ordinary alarm clock mechanism (not shown). This clock gear turns constantly at a uniform rate, and at regular intervals it permits one of the trip-wheel teeth to slip between two of its own, the next trip-wheel tooth coming to rest on the succeeding clock-gear tooth.

The details of the operation and construction of the timing mechanism are shown in the projected views at the extreme right of the diagram. In position 1 tooth A on the wheel *TW* is about to slip between two teeth on the constantly moving gear *G*. When it does so the valve turns one quarter-turn and tooth B comes to rest on tooth 1 (position II). When tooth 1 moves from under tooth B, the latter slips through slot 2 and tooth C comes to rest on tooth 1. Likewise, when tooth C passes through slot 2, tooth D rests on tooth 2, and so on.

*From the Laboratories of Physiology in the Harvard Medical School.
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†Medical Fellow of the National Research Council.

Actually, therefore, the administration consists of intermittent spurts. With a sixty-tooth clock gear they occur every thirty seconds inasmuch as each of the sixty teeth permits two quarter-turns of the valve. The frequency could be varied, of course, by the use of different clock gears. The accuracy of the clock is not altered by the friction between the gears, even when twice the weight necessary to turn the valve is employed. By means of multiple pulleys and a proportionally heavier weight the apparatus can be made to operate at least twenty-four hours without rewinding, and even then the string can be rewound without interrupting the delivery by disengaging the spool and rotating the valve by hand while rewinding.

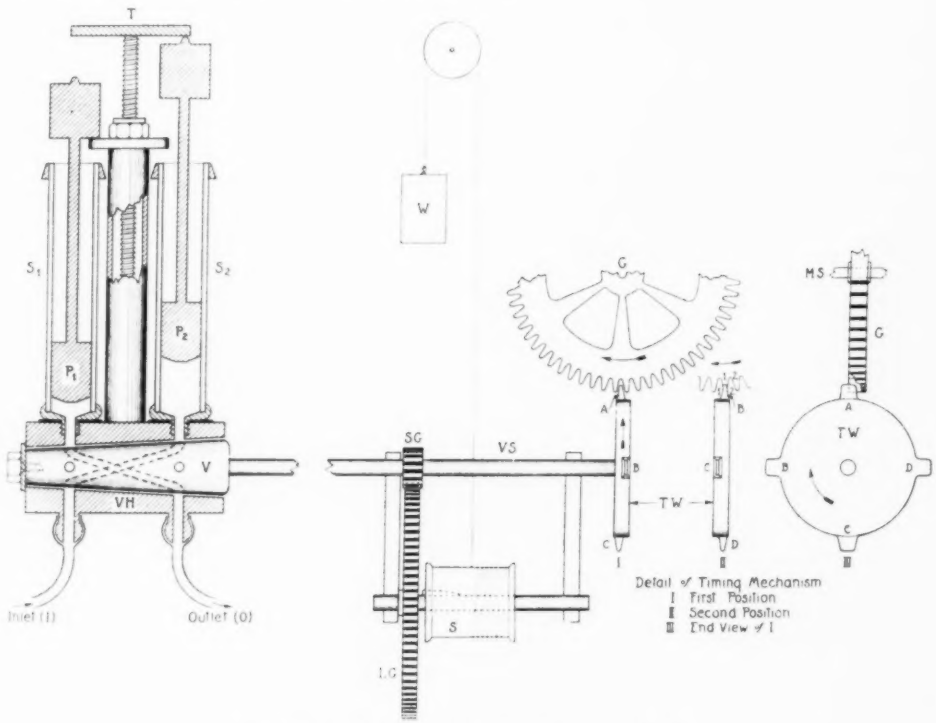

Fig. 1.—Apparatus drawn semidiagrammatically.

The top stop for the pistons is adjustable to different heights, the length of the stroke and therefore the delivery per hour being determined by the height of the stop. A vernier millimeter scale permits the stop to be set accurately at any desired height and hence for any predetermined delivery rate after the apparatus has been calibrated. Using 1 c.c. Record syringes and a sixty-tooth gear on the clock shaft, the apparatus will permit measured administration rates within a range of 1 to 150 c.c. per hour; and by using syringes and gears of different sizes, a much wider range could be obtained if necessary. The actual delivery in any experiment may be checked easily by suspending the solution in a graduated burette. In a long series of experiments involving different rates of administration the observed delivery has never varied from that estimated by more than 1 per cent.

The lubrication of two parts deserves particular mention. The pistons operate more freely and leaking above them is negligible if they are lubricated with a light grade of sperm oil. W. F. Nye's watch oil is ideal for this purpose. The valve can be made entirely water-tight by means of rubberized vaseline, prepared by melting one part of rubber tubing into about ten parts of vaseline and one part of light machine oil. Grinding and fitting the valve is the only difficult process in the construction of the apparatus. Fitting a valve which will operate freely and yet not leak requires patience and a fair degree of skill. Once suitably fitted, however, and provided with proper lubricant and motive force, it does not wear appreciably. Occasional leaking or jamming is always the result of inadequate cleaning or lubrication.

Inasmuch as the injecting force is limited to about 200 cm. of water, it is true that any complete obstruction of the venous cannula will interrupt the injection. In this respect Woodyatt's apparatus is superior, especially for use with conscious animals. Yet, scrupulous cleanliness of this apparatus and care to prevent clotting and twisting of the cannula is all that is required to avoid any obstruction whatsoever. In the use of anesthetized animals or perfusion preparations this feature presents no difficulties. Intraarterial administration to the normal animal is scarcely possible, but perfusions at constant rates could be performed admirably. In fact, with slight changes the instrument could easily be modified to duplicate the action of the cardiac ventricle in force and frequency.

The original apparatus has given dependable service in well over a hundred experiments for an aggregate of about thirteen hundred hours. It has frequently operated through the night with no attention, the longest uninterrupted injection occupying forty-eight hours. It was made in the machine shop of Harvard Medical School under the supervision of Mr. F. J. Christensen, whose cooperation is acknowledged gratefully.

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METHOD OF MOUNTING PATHOLOGY SPECIMENS UNDER WATCH CRYSTALS AND USING A COLORED BACKGROUND FOR CONTRAST*

By C. H. Manlove, M.D., Portland, Oregon

THE method of mounting specimens under a watch crystal has been used for a number of years. My attention was called to this method by the work of Day.[1]

The technic I wish to describe makes the mounts more durable, adds to their attractiveness for exhibition in the museum, and improves the visibility of the pathology one wishes to present. It is particularly adaptable to mounting such specimens as sections of the brain, heart, kidney, and other organs. It is also suitable for mounting the smaller structures as thyroid, throat organs en masse, and uterus.

Briefly, the technic consists of mounting a specimen beneath a watch crystal (*C*), using a flat piece of glass as a base, and securing the crystal in place by the use of some adhesive substance; filling the crystal with fluid, and then utilizing, as background, some color which will enhance the natural color of the tissue and more clearly demonstrate the pathology desired.

I have found several factors essential to the success of this method and enumerate these as follows:

In the first place, to secure good coloring of the tissue itself the specimen is treated, as soon as possible after removal, by placing it in Kaiserling Solution No. 1, through which illuminating gas is bubbled for several hours. The carbon monoxide content of the gas is the essential ingredient, as this combines with the hemoglobin to form a red carbon-monoxide hemoglobin compound. It is essential that all the Kaiserling solutions be kept saturated with this gas. The specimen is then kept in a tight jar in the same Kaiserling Solution No. 1 for at least twenty-four hours. The length of time in the No. 1 solution depends on the amount of fixation required, after which it is quickly washed in water to cleanse the specimen. It may then be placed in alcohol for a short time. This is not necessary, and apparently makes no marked change in the color content. The specimen is then transferred to Kaiserling Solution No. 3, which has been thoroughly saturated with illuminating gas, to which I add approximately 5 per cent of Kaiserling Solution No. 1. The addition of Kaiserling Solution No. 1 prevents mold and also prevents tissue such as intestine and brain from becoming translucent.†

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†The water used in the preparation of the Kaiserling solution is practically the equivalent of distilled water.

In order to preserve the color it is essential that the specimen be mounted as early as possible and hermetically sealed. I have specimens with excellent color that have not been out of Kaiserling No. 1 solution for over a year and still show no depreciation in color. It is apparent from this that the prevention of oxidation is the keynote to maintaining color.

Plate glass is used for a base (see Fig. 1, *A* and *B*) which must be heavy enough, so that it will remain practically rigid when handled. For crystals up to 8 inches diameter the glass base should be not less than ¼ of an inch in thickness. The plate should measure at least one inch greater in width than the diameter of the watch crystal used. The glass base *(A)* should have a

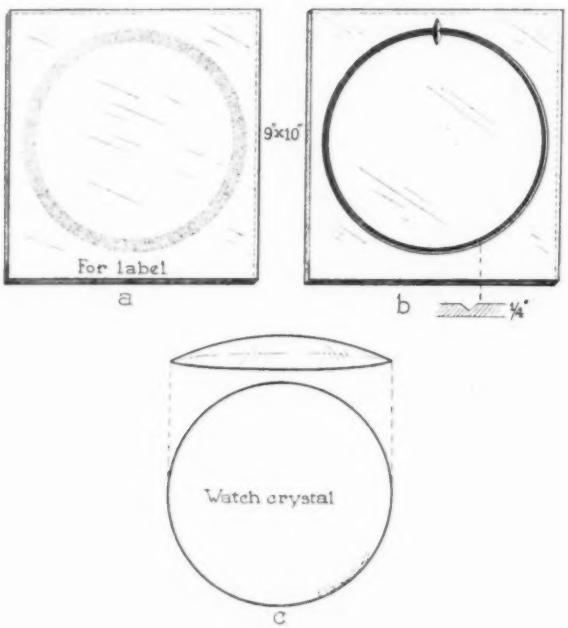

Fig. 1.

circular pathway of ½ inch width ground on its surface, to be of such diameter that the periphery of the watch crystal used will rest in the center of the pathway. The pathway should be placed in an eccentric manner on the glass base so that space will be allowed for labelling the specimen. One can take a plain glass base and with a rough emery stone roughen a pathway which acts almost as well as one ground by machine.

I have recently designed a glass base which has a moat ground into it *(b)*. This moat is made to fit the watch crystal. A short cross moat is constructed for filling the crystal with fluid. Fig. 1 illustrates the types of bases and the watch crystal.

The specimen to be mounted should be cut to the proper thickness, and a diameter that will allow it to fit snugly beneath the crystal so that it will not move when the mount is completed. The size should be approximated

when first placed in Kaiserling No. 1 solution and later a fresh surface cut. This latter should be made before complete fixation of the specimen.

In order to seal the watch crystal to the glass base I use a material designated as a seam filler.* This material has stood up for several years and is much better than ordinary tar. It withstands considerable heat without becoming fluid. The method of applying it is all important to insure success. The glass base and watch crystal must be absolutely dry. The seam filler is melted over an open flame. I use a large ladling spoon and melt about one

Fig. 2.

fluid ounce of the material at a time. This is then poured onto the ground pathway of the glass base while as liquid as it can be made. This is essential to make it adhere properly to the base. Allow this to cool and then add several layers with semiliquid seam filler. A dam should be constructed at least ¼ inch in height. Now apply flame to the dam to insure again its sealing of the inner surface to the glass base and also to smooth the dam. One may also use a mold and then allow the seam filler to cool and remove mold by applying heat to mold.

*Nonpareil Seam Filler. Manufactured by the Armstrong Corporation, Pittsburgh, Pa.

A 22 gauge hypodermic needle is now embedded into the dam of seam filler so that it extends through to the inner part of the dam.

The specimen which is to be mounted and which has been cut and prepared to fit beneath the crystal is sponged with a dry cloth to remove all excess fluid and placed inside the dam on the glass base.

Fig. 3.

Gentle heat is applied to the dam of seam filler and the watch crystal to be used is pressed firmly into it. This can be successfully accomplished by applying heat to the edges of the watch crystal while continuous pressure will gradually force the crystal into the dam until the crystal comes in contact with the base, and a rim of seam filler has welled up the inner sides of the crystal for at least one-half an inch. Now allow the mount to cool, and keep enough weight on the crystal to hold it in place although there is little danger of it moving if the specimen beneath has been properly prepared. The

heat applied to the crystal should not be such as to injure the specimen. I have never had this occur even though the heat has frequently broken the crystal.

After cooling remove by means of a hot spatula or putty knife all the excess seam filler from the outside except that in immediate contact with the outside half of the ground pathway. Then clean base outside of pathway with xylol.

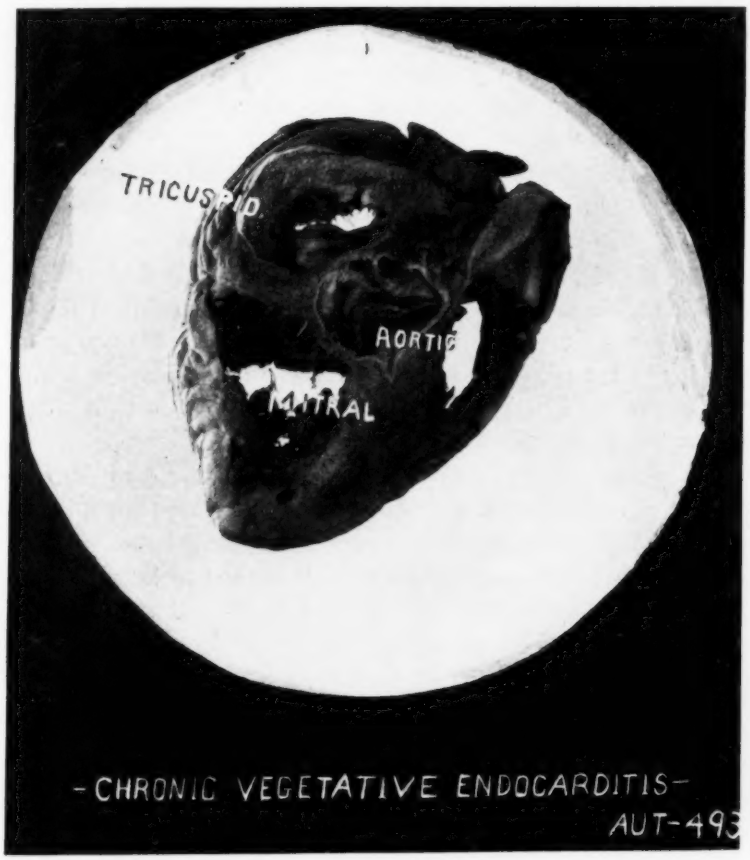

Fig. 4.

Now apply a rim of the seam filler to the outside edges of the watch crystal. To apply this so that a smooth even rim is formed, heat the seam filler and allow to partially cool, then pour slowly while holding the entire mount and rotating so that the material runs evenly around the entire circle of the crystal. If the material is a proper temperature this may be placed so that it is smooth and fairly even. It may then be smoothed with a hot spatula.

Again allow the mount to cool and then begin to introduce the Kaiserling Solution No. 3 through the 22 gauge needle, placed as mentioned. This needle,

in the meantime, has acted to allow a free flow of air into and out of the chamber beneath the crystal. The fluid is introduced by means of a 10 c.c. syringe by alternately injecting a few c.c. of fluid and then withdrawing a similar amount of air. If too large an amount of fluid is forced into the chamber under pressure, it will cause separation of the crystal from the base. Continue to fill the chamber until a fairly good sized air bubble remains. At this time remove the needle by rotation and traction. The remaining bubble of air is removed by placing the specimen beneath water or under a running faucet and then by gently pressing on the crystal, force out the air and allow water to completely fill.

While the mount is still beneath water, take a match or a wooden applicator and gently tampon some of the seam filler into the opening left when the needle was removed.

There is now left the problem of completing the external rim of seam filler. The specimen should be thoroughly dried, especially where the needle opening was placed. I usually remove about 1 inch of the external rim of seam filler on each side of the needle opening and after thoroughly drying this area apply the seam filler as before. Allow it to cool and smooth with spatula. It is necessary to use much care in this last step or a leak may occur. If the seam filler is applied when too hot it will melt that which was tamponed into the needle opening. Finally smooth the external rim with gentle heat and clean the surrounding glass with xylol.

After complete cooling, apply a coat of black enamel to the external rim of seam filler and also paint the back of the glass base except for the area covered by the watch crystal.

To place the label, I find white golf ball enamel to be excellent material. It is necessary to thin it to writing consistency. For this, xylol may be used. The label may be placed with a printing pen or by use of a stencil. This enamel will dry within a week so that the glass may be dusted or washed without danger to the label. (See Figs. 2, 3, and 4.)

The attractiveness of the mounts and the visibility of the specimens are much improved by the use of colored backgrounds. This is accomplished by the use of colored cardboard which is placed back of the mount so as to form an outline of the specimen, giving a contrasting or enhancing color to the space within the watch crystal that is not taken up by the specimen.

I have found that orange and deep green are the most commonly suited colors. However, these do not have to be in contrasting shade to the specimen, because in many I find that the orange will markedly improve a tissue with considerable red color of its own. The orange is of much help in displaying tissue with but little color. The green is of most help with tissues which have a deep, bright red. Other colors such as yellow, dark red, light green, and occasionally a bluish green will suit a small number of the specimens. These colors are selected by a process of elimination, by trying first one, then another.

The use of the color scheme with the base painted black, as de-

scribed, and altogether mounted on a black bench presents a museum display that is pleasing to look at and certainly increases the visibility of gross pathology.

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Good Samaritan Hospital.

A QUANTITATIVE TUBERCULIN TEST*

By John E. Blair, Ph.D., and Walter I. Galland, M.D., New York City

THE diagnosis of tuberculous lesions, particularly joint tuberculosis, manifestations of which may be obscure, is as a rule not clarified by the classical von Pirquet test. It is generally accepted that this diagnostic method is of little use except in the period of infancy and very early childhood, when a positive von Pirquet is presumptive evidence of an active tuberculous infection. Soon after, most individuals develop some degree of tuberculin hypersensitivity because of latent or previous infection sufficient to produce a cutaneous response to the usual scarification tuberculin test. Such reactions are as a rule of no clinical significance, and for this reason diagnosis by the aid of tuberculin in the form of the von Pirquet test has been largely abandoned as a useful procedure, except in the early years of life.

Since the von Pirquet test is purely qualitative, attempts have been made to develop a quantitative tuberculin test. These tests, which have been summarized by Hamman and Wolman of Johns Hopkins,[1] have for the most part proposed some modification of the von Pirquet technic, in which varying dilutions of tuberculin have been applied to the scarified skin, the sensitivity of the patient being subsequently estimated by the size of the wheal at the site of application of each dilution. The results in general proved to be of no more value than the ordinary von Pirquet.

The Mantoux test consists of administering OT. intracutaneously in a dilution of 1:10,000. Sensitivity to the tuberculin in this dilution is considered a reaction of diagnostic value, but as no series of dilutions is used, we believe that it has no greater value than the ordinary von Pirquet. Hamman and Wolman proposed an intracutaneous quantitative test, based on the Mantoux reaction, in which they employed three intracutaneous injections of 0.05 c.c. of OT. diluted to 1:10,000, 1:100,000, and 1:1,000,000. Smith[2] used a similar test, but carried the dilutions to 1:10,000,000. We have been unable to find a report of the application of either of these tests to an extensive series of cases.

Atsatt,[3] working at the Children's Hospital and the Massachusetts General Hospital in Boston, described in 1927 a modification of the Mantoux intracutaneous test, which he considers to be of value in the diagnosis of bone and joint tuberculosis. The essence of his quantitative reaction consists of establishing a threshold of tuberculin sensitivity, above which threshold tuberculin allergy would warrant a presumptive diagnosis of clinically active tuberculosis, but below which threshold one might expect various degrees of tuberculin allergy to be exhibited by persons carrying latent or healed tuberculosis. Using Saranac human tuberculin, a dilution of 1:7500 was established as the threshold of reaction. The test as he describes it, consists of administering, at

*From the Laboratory Division, Hospital for Joint Diseases.
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one session, intracutaneous injections of 0.1 c.c. of Saranac tuberculin in dilutions of 1:1000, 1:2500, 1:5000, 1:7500, and 1:10,000, together with a saline control. A cutaneous response to the 1:10,000 dilution is considered as being significant of the presence of clinical bone or joint tuberculosis, in the absence of other tuberculous manifestations. He reports a series of tests covering 211 cases, 85 of which were tuberculous, upon whom this quantitative reaction was performed. Considering his cases, irrespective of age groups, 90 per cent of the nontuberculous cases gave a negative reaction, while 92 per cent of the tuberculous cases gave a positive reaction, that is, positive in the dilution of 1:10,000.

Stimulated by the work of Atsatt, we attempted to repeat his quantitative test on cases in the various services of the Hospital for Joint Diseases, using tuberculin furnished by the New York Department of Health. Following the technic described by Atsatt, we performed the test upon 11 patients exhibiting a variety of bone and joint diseases, but obtained conflicting results. Our greatest difficulty was with cases known to be clinically nontuberculous, in some of whom we obtained florid positive tuberculin reactions with the dilutions proposed by Atsatt. However, we were under the definite impression that the quantitative intracutaneous test might have usefulness. Inasmuch as we obtained florid reactions in nontuberculous cases in the 1:10,000 dilution, we thought that the tuberculin we used might be more potent than the Saranac tuberculin employed by Atsatt.

Consequently we continued our investigations, but used dilutions of 1:10,000, 1:25,000, 1:50,000, 1:75,000, and 1:100,000 of the New York Department of Health tuberculin, made up in normal salt solution to which 0.5 per cent phenol was added as a preservative. Using these dilutions in a similar manner to that described, upon a series of 144 cases, covering all age groups and including tuberculous and nontuberculous individuals, we concluded that a positive local reaction in dilutions of 1:10,000 and 1:25,000 was obtainable in many clinically nontuberculous cases, and that response to these dilutions had no constant diagnostic value. However, positive reactions to dilutions above 1:25,000 were subsequently proved to have considerable diagnostic significance. These results showed in addition that the Mantoux test, as performed in this city, could be misleading, since the Department of Health, using the same tuberculin, considers reaction to the 1:10,000 dilution as significant. We have therefore established the dilution of 1:25,000 as the critical threshold in the quantitative test as we perform it. In practice, the critical threshold for each lot of tuberculin used may easily be determined by a few tests on tuberculous and nontuberculous cases, and dilutions may then be prepared sufficiently high above the threshold to give significant results.

In order to explain the different thresholds proposed by Atsatt and ourselves, we prepared dilutions of Saranac tuberculin from 1:10,000 to 1:100,000, and used the Saranac and New York Department of Health tuberculins in parallel series on the same cases. We concluded that the Saranac tuberculin was distinctly less potent than the Department of Health preparation. This may account for the difference between our critical threshold and that used by Atsatt. We feel, however, that it is preferable to use a tuberculin admitting of high dilution in order to reduce the absolute quantity of tuberculin

protein administered. A dilution of 1:1000 of many of the tuberculins obtainable appears to be too concentrated to permit of its routine use in tuberculous subjects.

The test in the completed form has consisted of the intracutaneous injection of .05 to 0.1 c.c. of the diluted tuberculin upon the volar surface of the forearm. A control injection of phenol-saline solution was also made. The tuberculin was injected in dilutions of 1:10,000, 1:25,000, 1:50,000 and 1:75,000 and 1:100,000. The dilutions were kept on ice and were discarded at the end of a week to ten days, at which time they were still potent. The syringes and needles were thoroughly cleaned prior to use. The tests were read at the end of twenty-four and forty-eight hours.

The maximum positive reaction usually appears during the first twenty-four hours, but we have noted a number of cases in which the development of the reaction was delayed, and did not reach its fastigium until the end of forty-eight hours. The positive reaction consists of the formation of an indurated cutaneous nodule at the site of injection, with or without a varying amount of erythema surrounding the nodule. Occasionally a bleb forms at the summit of the nodule in the more florid reactions. We would particularly note that we consider induration as the most important criterion of reaction. We have frequently observed erythema in sensitive skins even with the phenol-saline control, and in colored patients, it is quite impossible to judge the presence of erythema. The induration is best detected by gently rubbing the finger over the volar surface of the arm at the sites of injection. A definite shotty nodular sensation is imparted. The size of the nodules obtained varies with the susceptibility of the individual, the character of the skin, the amount of fluid injected, and possibly other unknown factors. Even when small, the induration is definite and unmistakable.

In reading the results, we consider as positive, and indicative of clinically active tuberculosis, those cases which show a reaction of induration in all dilutions, except the saline control. Occasional cases have exhibited a reaction up to and including the 1:50,000 dilution, and have been negative above that. We have considered these cases as negative. We are under the impression that such reactions indicate a variation from the threshold. We feel that the use of several dilutions, well above the critical threshold, gives a sufficiently wide margin for the occasional variation, and at the same time the higher dilutions will have diagnostic value.

In the 144 cases included in this report, both tuberculous and nontuberculous adults and children are represented. We have attempted to confirm the results of the quantitative reactions whenever possible, either by guinea pig inoculation, direct smear, x-ray, or pathologic examination of tissue.

We can summarize our results as follows: Of 78 cases of all ages finally considered as tuberculous, 71, or 91 per cent, gave a positive reaction in all dilutions, and 7 cases, or 8.9 per cent, gave an entirely negative reaction. Of 66 cases finally considered to be nontuberculous, 60, or 90.9 per cent, gave a negative reaction which was of diagnostic significance, while 6, or 9 per cent, gave a positive reaction in all dilutions.

The failure to obtain a positive reaction in approximately 9 per cent of tuberculous patients does not, however, invalidate the usefulness of the pro-

cedure. It is a known fact that in patients suffering from an overwhelming tuberculous infection, or in patients exhibiting but little resistance to the progress of the disease, there is frequently a total absence of tuberculin allergy, and though the allergic response is negative, there are other criteria for making a positive diagnosis. Such patients are considered as having a poor prognosis. Furthermore, such conditions as sepsis, intercurrent infections, and anemia may result in a lowering of tuberculin allergy. Among our tuberculous cases exhibiting negative reactions, there were 2 patients who died within a short time after we saw them, and 2 patients who are rapidly succumbing to the disease. At the time we read the reactions on these patients we stated that we considered the prognosis unfavorable. A fifth case of tuberculosis with a negative reaction was a patient who had previously received a rather protracted course of tuberculin therapy, and was therefore rendered tuberculin immune to the minute doses used. This leaves but two patients to be accounted for, and we have not been able to follow them up.

In regard to the 9 per cent of positive reactions in patients, the clinical lesion of which was finally considered nontuberculous, all but 2 of these 7 were adults, and we are by no means certain of the absence of some undiscovered focus of active tuberculosis. It is such cases that give us the most trouble, and it is these cases which emphasize the need of utilizing every available procedure for the diagnosis of tuberculosis.

It is difficult to consider each of the 144 cases tested in detail. We will confine ourselves to a few cases which will perhaps serve to demonstrate the possible clinical value of quantitative tuberculin reactions in establishing a diagnosis.

CASE 85.—L. I., male, aged twenty-nine. Admitted to the hospital with diagnosis of tuberculosis of the knee. The patient had suffered several years with an infection of the knee, which had been treated in several other hospitals, and which had been diagnosed as tuberculosis. The reaction obtained in this case was negative in all dilutions above 1:10,000, but positive in this dilution and in the von Pirquet. The result of this test was reported as negative. The patient was operated upon; the popliteal swelling was incised, and a gumma was found. The Wassermann was 4+.

CASE 94.—E. D., male, aged fifty-seven. Admitted with the diagnosis of tuberculosis of the knee. Four months before admission he had a swelling of the left hand and right knee. The patella was floating, and the motion of the knee was limited. A clinical diagnosis of tuberculosis of the right knee was made. The x-ray examination showed a moderate thickening of the apical pleura, which was reported as probably a healed tuberculosis. The quantitative tuberculin reaction was negative in all dilutions. An arthrotomy was performed, and the tissue which was removed from the knee was reported as chronic nonspecific synovitis, nontuberculous.

CASE 22.—A. S., male, aged seventeen. Admitted to the hospital with diagnosis of epiphyseolysis. While in the hospital, he developed a pleural effusion of unknown origin. Guinea pigs inoculated with the pleural fluid were positive for tuberculosis. Previous to the development of the effusion,

the quantitative test had been performed, and it gave a positive reaction in all dilutions.

CASE 13.—L. B., male, aged thirty. Admitted to the dispensary with diagnosis of old osteomyelitis of the femur and ankylosis of the knee joint. The x-ray diagnosis was old destructive arthritis of the knee, probably gonorrheal. This patient was treated in the dispensary on the assumption that it was gonorrheal ankylosis of the knee. The quantitative test revealed a florid reaction in all dilutions. A small area of fluctuation was detected on the medial aspect of the knee, and aspirated. Guinea pig inoculation of the aspirated fluid was positive for tuberculosis.

It is unnecessary to comment upon these cited cases, except to state that the use of the quantitative tuberculin reaction has in a number of instances led to the subsequent reversal of the original diagnosis.

In all tuberculin work the objection has been repeatedly urged that the administration of tuberculin frequently engenders an exacerbation of the tuberculous process, and a dissemination of the infection. One case in our series developed tuberculous meningitis about a week after the performance of the quantitative test. We are not inclined to attribute this complication to the tuberculin administered. The case in question was a child, five years of age, admitted with a diagnosis of tuberculosis of the hip, who gave an entirely negative quantitative reaction in all dilutions, and was recorded as having a poor prognosis. Two days following the administration of the quantitative test, a diagnosis of miliary tuberculosis was established, but in retrospect the child had shown evidences of meningeal involvement even before the test was made. We do not believe that the dissemination of tuberculosis in this child resulted from the intracutaneous injection of the extremely minute amount of tuberculin present in the dilution we used. Furthermore, since this child was insensitive to tuberculin, how could the injection of dilute tuberculin lead to a dissemination of the infection? We feel that the intracutaneous use of tuberculin, even in fairly large amounts rarely results in a focal or general reaction. The Mantoux test, which is quite widely used, involves the administration of 1/100,000 c.c. of OT. The test as we perform it, uses a total of about 1/50,000 c.c., which we feel is an infinitesimal dose, and is quantitatively incapable of producing any focal or general reaction, particularly as this is injected intracutaneously. In addition, in our series of 144 cases, in which the skin was always carefully prepared before injecting the tuberculin dilutions, there has not been a single instance of infection or other unfavorable results referable to the administration of the quantitative test.

The question may be fairly asked whether the quantitative tuberculin reaction gives any more information than the classical von Pirquet. Practically all of these cases have been controlled either by the von Pirquet or by a modification of that test. In forty cases in which the von Pirquet technic was used, the reaction of the latter in nontuberculous patients was comparable to that obtained with the dilutions below our critical threshold. These comparative reactions would signify that frequently the von Pirquet response may be positive in cases in which the quantitative test would establish as definitely negative. It has been shown that in nontuberculous individuals over ten to fifteen

years of age, positive von Pirquet or Mantoux reactions may be obtained in from 55 per cent to 89 per cent of cases, as cited by Calmette.[4] In our small series of cases of nontuberculous individuals over ten years of age, the number of positive reactions below the critical threshold approaches von Pirquet's own figures. However, it appears to be of significance that we have obtained 91 per cent of diagnostically significant negative reactions in these nontuberculous patients.

We would conclude by stating that we consider the quantitative intracutaneous tuberculin test as a valuable accessory in the diagnosis of active tuberculosis in all age groups. We do not consider the test infallible, but we feel that its accuracy is comparable to that of many of the commonly accepted serologic and immunologic diagnostic reactions. The reaction must be considered in conjunction with all other available clinical and pathologic data. Intelligently used, it will perhaps aid in establishing a final diagnosis in many obscure cases, particularly in adults.

SUMMARY

A quantitative tuberculin test has been elaborated, which is based upon a similar test proposed by Atsatt, but has been modified to give an apparently greater differentiation between clinically active tuberculosis and latent tubercular infection.

The test consists of intracutaneous injections of 0.1 c.c. of OT. diluted with phenol-saline to 1:10,000, 1:25,000, 1:50,000, 1:75,000 and 1:100,000, together with a saline control. Reactions are read after twenty-four and forty-eight hours. A positive reaction consists of the formation of an indurated cutaneous nodule, with or without erythema, at the site of injection. With the tuberculin used, a critical threshold of 1:25,000 was established, above which threshold positive reactions were considered as presumptive evidence of clinically active tuberculosis. Positive reactions below the critical threshold, with no reaction above the threshold, appear to indicate latent tubercular infection or healed lesions.

In a series of 144 cases, including tuberculous and nontuberculous individuals of all age groups, 91 per cent of the tuberculous cases gave a positive reaction in all dilutions, while 90 per cent of the nontuberculous cases gave a diagnostically significant negative reaction.

When intelligently performed, and used in conjunction with all other available clinical and pathologic data, the test appears to have value as an accessory in the diagnosis of clinically active tuberculosis.

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1919 Madison Avenue.
1085 Park Avenue.

APPLICATION OF THE CORPER-UYEI CULTURE METHOD IN THE ROUTINE EXAMINATION OF SPUTUM FOR TUBERCLE BACILLI*

By John Sumter Cunningham, M.D., and Priscilla Lees Cummings, B.S., Rochester, N. Y.

RECENTLY there has appeared a new method[1] of cultivation of tubercle bacilli from contaminated materials which has been claimed by Corper[2] to be superior to other culture methods and to the smear tests, and of equal efficiency and more advantageous than the guinea pig inoculation method[3] for the laboratory diagnosis of tuberculosis. In view of the demonstrated relative uncertainty of the ordinary stained smear for the detection of tubercle bacilli in sputum[2] this new culture method suggested a study to determine the efficiency of the old routine smear examination as compared with sulphuric acid-crystal violet-potato medium culture method, with a view to its possible adoption as a routine procedure in the examination of sputum in the public health laboratory. Accordingly, a series of specimens of sputum during a seven-months' period was subjected to culture according to the Corper-Uyei technic in addition to the regular (carbolfuchsin stained) smear examination; and the cultures thus obtained were injected into guinea pigs to determine the pathogenicity of each strain of tubercle bacillus isolated.

METHOD

When a specimen of sputum arrived at the laboratory, it was smeared, stained, and examined. As soon as practicable a culture was made. At times it was necessary to store the sputum in the cold room as long as thirty-nine days after being delivered to the laboratory for diagnosis, before it could be cultured. This storage period, at about 4° C., did not appear to affect the viability of the tubercle bacilli, as evidenced by growth subsequently on the crystal violet-potato medium. The average period from the date of collection of the sputum from the patient until its cultivation in the laboratory was approximately seven and five-tenths days.

1. Smear.—The routine smear examination consisted of selecting a purulent fleck of sputum (if present), smearing it uniformly on a clean glass slide, fixing it in a flame, staining by the Ziehl-Neelson carbolfuchsin-acid alcohol-methylene blue counterstain technic, and then examining the smear microscopically for acid-fast bacilli. Careful microscopic search was maintained for at least five minutes before a report of a "negative smear" was made; and the detection of a minimum of 8 acid-fast rods resembling tubercle bacilli was the requisite for a "positive smear" diagnosis. When one or more acid-fast bacilli were detected during the minimal five-minute period, the examination

*From the Department of Bacteriology and the Rochester Health Bureau Laboratories, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

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was prolonged indefinitely in an attempt to discover the number of organisms necessary for a positive diagnosis.

2. Culture.—The method of culture is essentially that described by Corper,[2] with minor modifications. For convenience the sputum was transferred from the standard cubical collecting bottle to a sterile Petri dish, then smeared and cultured immediately, or placed in the cold room for later cultivation. One c.c. of unhomogenized sputum (in some instances less was available) was transferred by means of a sterile pipette into a sterile, graduated and corked 15 c.c. conical centrifuge tube, and an equal quantity of 6 per cent sulphuric acid (by volume) added. A thorough mixture was obtained by beating the contents of the tube with a sterile glass rod having an enlarged, roughly button-shaped end. The stirring rod was gently and rapidly oscillated to and fro in the mixture for about a minute, the impingement of the button-shaped end into the converging conical end of the centrifuge tube insuring a fine dispersion of the particles of sputum. In this manner the acid was allowed to permeate the sputum and exert its bactericidal effect on contaminating organisms present. Many such contaminants, found frequently in sputum, such as yeast, spores, staphylococci, streptococci, and Aspergillus niger will be eliminated if special care is taken to obtain adequate mixing of the sputum and acid at this point in the procedure. The centrifuge tube was then stoppered and incubated at 37° C. for half an hour. The contents were next diluted with about 10 c.c. of sterile 0.85 per cent sodium chloride solution, mixed well by inversion of the tube several times, and centrifugalized for five minutes at a moderate rate of speed. The supernatant fluid was decanted and a generous portion of the residue planted on the slant surface of the crystal violet-potato medium. Then the cotton plug, impregnated in its lower half with hot vaseline-paraffin mixture, was inserted into the tube and allowed to cool and harden. Finally the plugged tube was capped with tin foil and incubated at 37° C. for a minimal period of ten weeks.

The medium is prepared as Corper[2] originally recommended, "by cutting large, clean peeled potatoes, free from surface defects, into cylinders about 3 inches long and ⅝ inch in diameter. The cylinders are halved [diagonally and] longitudinally and immediately soaked in 1 per cent sodium carbonate solution containing 1:75,000 or 0.0015 per cent crystal violet (the dye and sodium carbonate should be mixed just prior to use to prevent decolorizing) for from one to two hours. After this the cylinders are gently wiped off with a clean towel, and are introduced into a sterile culture tube (6 × ¾ inch size) containing 1.5 c.c. of 5 per cent glycerol broth, cotton plugged, and are sterilized in an autoclave at 15 pounds pressure for at least thirty minutes."

3. Guinea Pig Inoculation.—As a check on the pathogenicity of the acid-fast bacilli obtained in each culture, guinea pigs were inoculated subcutaneously with 1 c.c. of an homogeneous suspension of 2 or 3 loopfuls of the growth in 2 c.c. normal saline. The acid-fastness of the organisms in the culture was determined beforehand, and in the event of a sparse initial growth, transplants were made on fresh medium to insure liberal stock cultures. The inoculated animals were weighed, placed in separate cages, and at the end of six to eight weeks were sacrificed by ether anesthesia, and autopsied. Anatomic tubercu-

lous involvement of the organs, especially the regional lymph nodes, spleen, liver and lungs was noted, and smears from these organs were examined for acid-fast bacilli.

RESULTS AND DISCUSSION

Contaminants.—The occurrence of contaminating organisms which overgrow the colonies of tubercle bacilli on the crystal violet-potato medium and which thus delay or inhibit the culture of some specimens, is an important factor in determining the practicability of the Corper method as a routine procedure in the public health laboratory. Table I shows the loss from contaminating organisms which may be expected when this culture method is introduced as a routine laboratory procedure, in the hands of anyone unpracticed in its technic, and during its development as a standard method for any particular laboratory.

By chance, during the first three months (September, October, November) when the percentage of contaminants was highest (averaging 13.5 per cent), these 26 contaminated cultures contained material from 5 specimens, from which smears were positive for tubercle bacilli. The examination for the growth of the acid-fast organisms on the culture medium naturally ceased with the overwhelming overgrowth of contaminants. Therefore, a fair comparison of these 5 positive smears with the same 5 potentially positive cultures is impossible. It will be seen in Table II, however, that this early advantage of the smear method is exactly compensated for by the same number of excess positive cultures. Referring to Table I again, it is apparent that the frequency of contaminations diminishes as skill is acquired, and in this instance as factors contributing to more frequent contaminations were eliminated. Thorough mixing of the acid and sputum is essential. At first a mortar and pestle were employed for this purpose, but this apparatus was too bulky and allowed too ready exposure to air contaminants. Accordingly button-shaped glass rods and conical centrifuge tubes were adopted[4] with a definite reduction in the percentage occurrence of extraneous organisms growing on the crystal violet-potato medium.

Contaminants in all instances in Table I appeared within the first week of cultivation. Since the tubes were not opened during this period, it is as-

TABLE I

CONTAMINATIONS IN ROUTINE CULTURES OF SPUTUMS FOR TUBERCLE BACILLI BY THE CORPER-UYEI METHOD

MONTH	TOTAL NUMBER OF SPECIMENS	TOTAL NUMBER OF TUBES	CONTAMINATED CULTURES		
			NO. DISCARDED	% DISCARDED	POSITIVE SMEARS
1928					
September	45	45	6	13.33	2
October	88	151	15	17.04	2
November	59	118	5	8.47	1
December	35	70	4	11.43	0
1929					
January	18	36	0	0.00	0
February	57	114	2	3.51	0
March	48	96	0	0.00	0
Totals	350	630	32	9.14	5

sumed that no invasion occurred from the air or through the vaseline-paraffined, tin foil-topped cotton plug, and that surviving contaminants were derived from the sputum or were air-borne only during the handling of the materials for culture.

Adequacy of Culture Compared with Smear.—A comparison of the efficiency of the smear and culture methods in the detection of acid-fast bacilli over the seven-month observation period is recorded in Table II. It is seen that of the 318 uncontaminated specimens of sputum cultured, 38 or 11.95 per cent were "positive cultures" and only 33 or 10.37 per cent "positive smears," a difference of 5. This difference represents an actual gain of 14.8 per cent above the 33 specimens classified as positive by the examination of the stained smear. Table III is an elaboration of Table II: on the basis of uncontaminated cultures of specimens the ratio of total disagreements is 10 "positive culture-negative smears" to 5 "positive smear-negative cultures," a total disagreement of 4.72 per cent. Thus in the month of October there was agreement in diagnosis of 95.88 per cent in 73 uncontaminated specimens (of sputum examined for acid-fast organisms by both methods) and a disagreement of 4.12 per cent, or in 3 specimens. Of these, all 3 specimens grew tubercle bacilli in culture, while no acid-fast bacilli were detected in the smear examination. On the other hand, almost the reverse was true in December when there were 2 "positive smears with negative cultures," and no "positive culture-negative smears."

TABLE II

COMPARISON OF THE ACTUAL NUMBER OF POSITIVE SPUTUMS OBTAINED BY SMEAR AND BY THE CULTURE METHOD

MONTH	SPECIMENS		NUMBER POSITIVE SMEARS	PER CENT POSITIVE SMEARS	NUMBER POSITIVE CULTURES	PER CENT POSITIVE CULTURES
	TOTAL	UNCONTAMINATED				
1928						
September	45	39	2	5.13	3	7.69
October	88	73	6	8.22	9	12.33
November	59	54	9	16.66	8	14.83
December	35	31	5	16.13	3	9.67
1929						
January	18	18	2	11.11	2	11.11
February	57	55	7	12.73	11	20.00
March	48	48	2	4.17	2	4.17
Totals	350	318	33	10.37	38	11.95

The importance of eliminating contaminants has been brought out, and is herewith reemphasized. Thus the gain of 5 or 14.8 per cent positive cultures (see Table III), representing the advantage of the sulphuric acid-crystal violet-potato medium method over the smear stained by the Ziehl-Neelson technic, is neutralized by the loss of 5 cultures by contaminants which were made from sputum showing acid-fast bacilli in the smear.

It is apparent that each method has both advantages and disadvantages. An increase in the number of positive diagnoses of tubercle bacilli in sputum specimens can be made by the employment of the culture method as a supplement to the examination of the stained smear in cases in which no acid-fast organisms are found in the smear.

TABLE III

VARIATIONS IN AGREEMENT OF DIAGNOSES BY SMEAR AND BY CULTURE (ON THE BASIS OF UNCONTAMINATED CULTURES)

MONTH	SPECIMENS		AGREEMENTS				DISAGREEMENTS			
	TOTAL	UNCONTAMINATED ON CULTURE	NEG. CULT. NEG. SMEAR	POS. CULT. POS. SMEAR	TOTAL	PER CENT	POS. CULT. NEG. SMEAR	NEG. CULT POS. SMEAR	TOTAL	PER CENT
1928										
September	45	39	36	2	38	97.44	1	0	1	2.56
October	88	73	64	6	70	95.88	3	0	3	4.12
November	59	54	43	6	49	90.74	2	3	5	9.26
December	35	31	26	3	29	93.55	0	2	2	6.45
1929										
January	18	18	16	2	18	100.00	0	0	0	0.00
February	57	55	44	7	51	92.74	4	0	4	7.26
March	48	48	46	2	48	100.00	0	0	0	0.00
Totals	350	318	275	28	303	95.28	10	5	15	4.72

In Table III it may be noted that in several instances stained smears of the sputum revealed acid-fast bacilli whereas in the subsequent culture on the crystal violet-potato medium no growth of tubercle bacilli was obtained. The explanation for the failure of these specimens to grow colonies of the organisms is not clear. Malkani[5] notes that a positive sputum may give growth on one occasion and at other times may not and admits his inabiltiy to offer an explanation. For the same reason, that he observes that ordinary cultures of tubercle bacilli when subjected to acid-digestion frequently grow out, and previously positive sputums do not result in growth by culture, it is possible that the latter instances are due to the presence of attenuated or weakened organisms or finely dispersed organisms (not in clumps as emulsions of cultures often are) which are more easily bathed in the acid and thus are retarded in growth or killed. It is possible also that the choosing of so-called typical purulent flecks for the making of smears favors the finding of acid-fast bacilli, whereas the random method of taking up 1 c.c. of sputum for the culture procedure may by chance not include such material and hence result in a negative culture.

At one stage in this series the temperature of the incubator rose to about 40° C. A number of cultures became partially desiccated, the 1.5 c.c. of 5 per cent glycerin bouillon even disappearing. This presents an uncalculable source of error against the culture method. Despite careful impregnation of the culture tube cotton plugs one-half with vaseline-paraffin mixture, a number of cultures became dry, even at 37° C., and these contribute further to this error. "Airing" of the cultures was carried out until the danger of contamination became apparent and until it occurred to us that adding fresh air to a bare culture medium surface did not contribute anything to the growth of organisms possibly present. It was, therefore, discontinued.

Ageing of the sputum may be another unfavorable factor, probably negligible, since all specimens were immediately stored in the ice box awaiting culture, thus preventing putrefaction due to contaminants. One batch of 30 specimens (in February) was kept overnight at room temperature; yet

these grew out 4 cultures that were negative sputum-smears, and only 2 contaminated specimens occurred in the lot.

One specimen of sputum in the series from a single patient is of particular interest since it grew out a single colony on the crystal violet-potato medium, while exhaustive examination of 10 different stained smears was fruitless in revealing acid-fast organisms. In 8 instances less than 3 colonies appeared on the potato slant from heavy inoculation of the acid-treated sputum. In only 3 of these specimens were acid-fast bacilli seen in the smear. The scarcity of growth probably explains why the organisms were missed in the smear examination in the majority of these sputums. The advantage of the culture in these specimens is apparent.

The earliest gross appearance of colonies of tubercle bacilli from cultured sputums occurred in fifteen days, while the average length of time required for all cultures was thirty-seven days. Examinations for new growth were made approximately every four or five days. Had it been possible to examine each culture every day it is probable that the average time of appearance would have been considerably reduced.

Cerebrospinal fluid obtained at autopsy from a case of tuberculous meningitis and untreated with sulphuric acid yielded an abundant growth on the potato medium in twenty-four days. Similarly, tuberculous lung tissues from human autopsy material and from guinea pigs readily grew tubercle bacilli when subjected to the usual technic of culture. It is suggested that suspected tuberculous exudates, obtained by an aseptic technic, be cultured directly on the crystal violet-potato medium as well as after acidification, as a doubly sure method of detecting the organisms.

Stock cultures of tubercle bacilli may be transferred and subcultured indefinitely on the crystal violet-potato medium, provided the culture material be kept adequately moistened. If allowed to dry the organisms soon become nonviable. The crystal violet does not seem to impair cultures maintained in this manner.

Pathogenicity of Cultures.—A point hitherto undetermined in reports in the literature on the application of the sulphuric acid-crystal violet-potato medium culture method is that relating to the pathogenicity of the acid-fast bacilli obtained from the sputum or other sources, after isolation on the medium.

TABLE IV

DISPOSITION OF CULTURES OF TUBERCLE BACILLI OBTAINED FROM SPUTUM BY THE CORPER-UYEI METHOD

Cultures dried; no growth on subculture; no animal inoculation	2
Cultures dried; old (2½-6 months); no growth on subculture (in one); animal inoculations twice negative	3
Cultures viable; animal inoculations successful in producing tuberculosis (acid-fast bacilli recovered in 30)	33
Positive cultures (acid-fast bacilli; characteristic colonies, etc.)	38

Table IV is a summary of the results of guinea pig inoculation of samples of the cultures obtained in the series. In all instances in which the organisms remained viable after isolation from sputum the subsequent injection of

these cultures into guinea pigs produced in six to eight weeks the picture of experimental tuberculosis, involving lymph glands, spleen, liver and lungs. Three cultures dried out considerably, so an attempt was made to stimulate them to grow by moistening them with glycerin bouillon added to each tube. Later at a culture age varying from eighty-five to one hundred and ninety-four days, finely divided suspensions of these organisms in saline were injected into 2 series of guinea pigs. In no case was there any macroscopic evidence of tuberculosis at necropsy.

SUMMARY AND CONCLUSIONS

A series of 350 specimens of sputum of which 318 remained uncontaminated was cultured over a period of seven months according to the Corper-Uyei sulphuric acid-crystal violet-potato medium method, in conjunction with the routine Ziehl-Neelson stained smear examination, to determine the comparative efficiency of each method in the laboratory diagnosis of tuberculosis. Thirty-eight positive diagnoses were obtained by the culture method and 33 by the smear examination, considering only those specimens which were not contaminated on culture. This gain of 5 extra "positives" or 14.8 per cent was exactly the same as the number of extra "positives" obtained on smear among the 32 contaminated specimens (see Table I).

To determine the pathogenicity of the acid-fast organisms derived from the cultures, guinea pigs were inoculated with suspensions of each growth. In every instance following the injection of viable organisms, tuberculous glands or organs were found at autopsy of the animals in six to eight weeks. Acid-fast bacilli were demonstrated in smears of the glandular pus or tissues in 30 of 33 animals.

Thorough mixing of the sputum and acid is essential to prevent the overgrowth of contaminating microorganisms in the cultures.

The sulphuric acid-crystal violet-potato method for isolating and detecting tubercle bacilli in the sputum, besides possessing the advantages previously pointed out by its originators,[3] is a more reliable means than the Ziehl-Neelson stained smear examination when the occurrence of contaminants is kept at a minimum; and when employed in conjunction with the smear test, it is a procedure of undeniable value in the laboratory diagnosis of tuberculosis.

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COMPARISON OF KOLMER, WASSERMANN, AND KAHN TESTS AND DARK-FIELD EXAMINATION IN PRIMARY SYPHILIS*

By Robert G. Owen, A.B., A.M., M.D., and Henry Erwin Cope, B.S., M.D., Detroit, Mich.

EXCEPT for isolated instances largely confined to the more recent reports, the literature on the Kahn test consists chiefly of statistical studies comparing its percentage of positivity with that of various types of complement-fixation tests. The past year or so has finally brought forth papers comparing the reactions obtained in the Kahn and Wassermann tests with the actual status of the individual patient with regard to syphilitic infection. We have found only four studies of the reliability of the Kahn test in primary syphilis comprising a total of 240 cases.

Keim and Kahn[1] report 47 cases of primary syphilis of which 17 were negative with both the Kahn and Wassermann tests (eighteen-hour ice box fixation); of the other 30, 27 gave strongly positive Kahn tests and three were weakly positive; 26 gave strongly positive Wassermann tests, one a weakly positive and three were negative.

Houghton et al.[2] report 38 cases of which 4 were negative with both tests, 34 positive with the Kahn test and 26 with the Wassermann test (time of fixation not given).

Willett and Nagle[3] in a study of the relative value of the Kahn test and dark-field examination in 105 cases of early syphilis report a total diagnostic accuracy of the dark-field examination in the first week of 76 per cent; the Kahn 56 per cent. In the second week the dark-field examination showed 60 per cent positive, the Kahn test 90 per cent, and in the third week the dark-field examination 50 per cent, and the Kahn 90 per cent. In the fourth week the dark-field examination gave 55 per cent, the Kahn test 88 per cent, while subsequent weeks showed the dark-field examination 25 per cent and the Kahn test 100 per cent positive.

McIntyre and Gilman[4] report 50 cases of primary syphilis with relative agreement between the Kahn and Kolmer tests in 83 per cent, absolute agreement in 68.7 per cent and no agreement in 17 per cent. Of the discrepancies 4.4 per cent had positive Kahn tests and negative Kolmer tests and 12.6 per cent positive Kolmer tests and negative Kahn tests.

We are reporting herewith a series of 1061 cases of proved early syphilis. All of the patients had dark-field examinations of the initial lesion, Kolmer blood Wassermann, and Kahn tests. In none of these cases does the diagnosis rest solely on clinical evidence. We saw 44 per cent of these patients in the

*From the Serological Department of The Owen Clinical Laboratory.
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first week of their infection, 28.4 per cent in the second, 12.2 per cent in the third, 4.2 per cent in the fourth, and 11.2 per cent in the fifth or subsequent weeks.

Of the total series, regardless of the duration of the lesion, 47.3 per cent showed positive dark-field examinations and negative serologic examinations, 35.1 per cent were positive both by dark-field and serologic examinations (Kolmer test, Kahn test, or both) while 14.6 per cent had negative dark-field examinations but positive serologic examinations. At the time of the first examination 3 per cent gave negative results with all methods, the diagnosis being made by subsequent tests. In other words a total of 82.4 per cent of these cases had positive dark-field examinations and only 49.7 per cent had positive blood tests.

The relative value of the dark-field examination becomes still more striking when these figures are analyzed by weeks. Of those patients seen in the first week after the appearance of the initial lesion, 71 per cent had positive dark-field examinations only; 21.5 per cent had both positive dark-field examinations and serologic tests, while 5 per cent showed positive serologic examinations only, a total accuracy of 92.5 per cent for the dark-field examination and 26.5 per cent for the blood tests. In the second week we found 45.5 per cent positive, only with the dark-field examination, 41.3 per cent with both dark-field and serologic examinations positive, and 9.3 per cent with positive serologic and negative dark-field examinations, a total of 87.3 per cent positive dark-field and 51.1 per cent positive serologic examinations. The third week gave 25.8 per cent positive with the dark-field examination only, 49.2 per cent positive with both tests and 21.9 per cent positive only with the blood tests, a total accuracy of 75 per cent for the dark-field examination and 71.1 per cent for the blood tests. By the fourth week we found only 4.4 per cent positive with the dark-field examination alone, 47.8 positive with both tests, and 41.3 per cent with serologic examination only; the accuracy of the dark-field examination being 52.2 per cent and the blood tests 89.1 per cent. In the fifth and subsequent weeks there were no patients with positive dark-field examinations and negative blood tests, 51.2 per cent having both tests positive and 47.9 per cent positive solely with the blood tests. The "misses" remain about 3 per cent throughout the entire first four weeks. It is therefore not until the third week that the serologic examination begins to approach the dark-field examination in diagnostic accuracy.

This series also represents an opportunity to study the relative sensitivity of the Kahn and Kolmer tests in a fairly large, controlled group of known cases of early syphilis. Tabulating these again by weeks we find, in the first week, 19 per cent positive with both the Kahn and Kolmer tests, 5 per cent positive only with the Kahn test, and 2.5 per cent only with the Kolmer test. In the second week there were 44.8 per cent positive with both tests, 3.3 per cent only with the Kahn test, and 3.0 per cent with the Kolmer test. In the third week 64 per cent were positive with both tests, 3.2 per cent with the Kahn test, and 3.9 per cent with the Kolmer test. By the fourth week 80.3 per cent gave positives with both tests, 8.8 per cent with the Kahn test only, and none with the Kolmer test (the relatively small number of cases in the fourth

TABLE I

SHOWING TOTAL NUMBER AND PERCENTAGE OF POSITIVE CASES WITH DIFFERENT METHODS AT VARIOUS PERIODS AFTER THE APPEARANCE OF THE PRIMARY LESION

DAYS SINCE APPEARANCE OF THE PRIMARY LESION	1-7 DAYS		8-14 DAYS		15-21 DAYS		22-28 DAYS		28-35 DAYS		TOTAL	
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Positive dark-field examination Negative serologic examination	330	71	137	45.5	33	25.8	2	4.4	0	0	502	47.3
Positive dark-field examination Positive Kolmer test Positive Kahn test	70	15	107	35.5	57	44.5	20	43.4	61	51.2	315	29.7
Positive dark-field examination Positive Kahn test Negative Kolmer test	18	4	10	3.3	2	1.6	2	4.4	0	0	32	3.0
Positive dark-field examination Positive Kolmer test Negative Kahn test	12	2.3	9	3.1	4	3.1	0	0	1	0.9	26	2.4
Negative dark-field examination Positive Kahn test Positive Kolmer test	17	4	28	9.3	25	19.5	17	36.9	57	47.9	144	13.6
Negative dark-field examination Positive Kahn test Negative Kolmer test	4	1.0	0	0	2	1.6	2	4.4	0	0	8	0.8
Negative dark-field examination Negative Kahn test Positive Kolmer test	1	0.2	0	0	1	0.8	0	0	0	0	2	0.2
Negative dark-field examination Negative serologic examination	15	3.5	10	3.3	4	3.1	3	6.5	0	0	32	3.0
Number and per cent of cases seen at different periods	467	44	301	28.4	128	12.2	46	4.2	119	11.2	1061	

week group gives these figures a fictitious accuracy). In the fifth week there was one patient who gave a positive only with the Kolmer test, the other 118 being positive with both tests. Altogether there were 33.3 per cent positives with both Kahn and Kolmer tests, 3.8 per cent positive with the Kahn test only, and 2.6 per cent positive with the Kolmer test only. So far there has seemed to be but little if any difference in sensitivity of the two tests provided all reactions of any degree are counted as positives. The question of the diagnostic significance of weak positives will be discussed later in this paper.

In this series there were 113 patients showing a marked discrepancy between the results obtained with the two tests. Of these, 47 gave a reaction with both the complement-fixation and precipitation methods differing markedly in degree, 31 having strongly positive Kolmer tests and weak Kahn tests and 16 strong Kahn tests and weak Kolmer tests. Of the 27 cases with positive Kolmer tests and negative Kahn tests, 11 were ++++ or +++ positive while of the 39 patients with positive Kahn tests and negative Kolmer tests, there were 14 with ++++ or +++ positive reactions, 25 being + or ++ in strength. The actual dependence to be put on such + or ++ positive Kahn tests becomes somewhat obscure when we find that in an additional 524 patients with venereal sores who were proved to be nonsyphilitic, there were 15 who showed at some time in the serologic investigation of the etiology of their infection Kahn reactions of varying strengths but who did not develop a positive Kolmer-Wassermann test within a period of more than thirty-five days after the onset of the lesion nor subsequent clinical evidence of syphilis. They were not treated for syphilis during this time. If the Kahn test alone had been taken as the diagnostic criterion, we would have had an incidence of false positive diagnosis in the negative cases of 3 per cent. The incidence of weak positive Kahn tests and negative Kolmer tests in the positive series is 2.5 per cent. Whether these aberrant positives represent true false positive reactions or technical errors, we are not attempting to say. These tests were run by experienced technicians, checked by one of us and all discordant results repeated. The cases reported here represent only those obtained on repeat tests. In the same group of cases we obtained one Kolmer test reading of 1-0-0-0-0 which proved not to be a case of primary syphilis.

CONCLUSIONS

1. The most reliable single diagnostic procedure in early syphilis is the dark-field examination.

2. Serologic examinations approach the dark-field examination in accuracy only after the third week of the primary lesion.

3. The multiple approach (dark-field and serologic examinations) gives the most information and the highest diagnostic accuracy (97 per cent).

4. In this series there is no appreciable increase in specificity nor earlier positivity of the Kahn test over the Kolmer-Wassermann method.

5. To obtain the highest technical accuracy both Kahn and Kolmer tests should always be made, checking both where a discrepancy occurs.

6. The Kahn test is more subject to technical error and weak positive results should not be taken as evidence that a venereal lesion is necessarily syphilitic.

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A TIME-SAVING DEVICE FOR WASHING TEST TUBES*

By Israel Davidsohn, M.D., and Elizabeth M. Yagle, Ph.D.,
Philadelphia, Pa.

WHENEVER large quantities of test tubes are used for laboratory procedures, for instance in the performance of the Wassermann test, their emptying and rinsing, preliminary to the thorough cleansing, is very time-consuming. The usual procedure is to take out with one hand about two or three test tubes at a time from the rack, to place them in the other hand until a handful of about six or eight have been collected, then to empty them by turning upside down, to fill them with water again, and to repeat that procedure as many times as necessary. To empty and rinse the test tubes of a rack holding about seventy-two tubes, the above procedure has to be repeated about ten times. Besides the loss of time, the handling of individual test tubes increases the breakage considerably.

To obviate the above difficulties, some laboratories use a rectangular piece of wire net, which is put on top of the filled test tube rack; it is held down on the sides with both hands and so the entire rack can be turned upside down

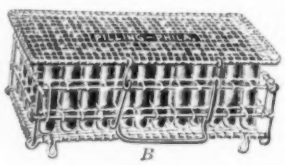

Fig. 1.

for the emptying and refilling of the test tubes. We have observed the use of such a wire net and tried it ourselves repeatedly. We noticed that the net has a tendency to slip and that a considerable amount of skill is necessary to handle it satisfactorily. If the procedure is not carried out properly, no time is saved; and the slipping of the net chips the edges of the test tubes. It occurred to us that the instability of the wire net could be removed by attaching it with two clamps to the shelf of the test tube rack.

As shown in the illustration, the apparatus consists of a rectangular net made of galvanized wire netting (A) with a broad wire clamp (B) on each side, catching at the middle shelf of the test tube rack. Any test tube rack provided with a middle shelf is satisfactory.

The apparatus permits turning the test tube rack with its contents upside down and emptying the tubes at once. The clamps hold the lid firmly and reliably. The apparatus is very easy to handle; it is simple in construction, and is readily made. The edges of the test tube mouths are not chipped, and the breakage is considerably decreased.

It is our experience that by the use of the above simple device valuable time is saved in a busy laboratory.

The George P. Pilling and Sons Company, Philadelphia, has cooperated in carrying out our designs.

*From the Laboratories of the Mount Sinai Hospital and of the Graduate Hospital of the University of Pennsylvania, Philadelphia, Pa.

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ATTEMPTS TO SECURE OBJECTIVE METHODS OF STUDYING MILD ANAPHYLAXIS*

By R. D. Templeton and W. F. Bollens, Chicago, Ill.

PHYSIOLOGIC means of recognizing a mild anaphylactic shock are not well developed.

Taking the guinea pig as the most susceptible animal Harvey and Templeton (1926) sought to determine the onset of anaphylaxis by observing the gross manifestations. Egg white was used as the foreign protein, and the time varied for the second injection from fifteen to twenty-four days after sensitization. If the shock was sufficiently severe, the gross symptoms were distinct, but in many instances the symptoms shown were not sufficiently different from the normal reactions of the control pigs to make this of diagnostic value. A few attempts were made to study the contractions of the descending colon, but with very little success.

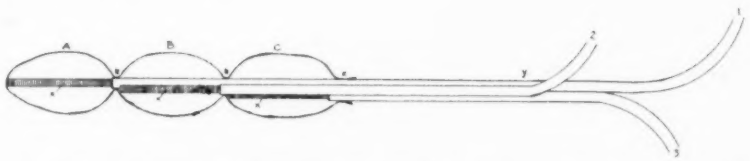

Fig. 1.—Triple balloon. *K*, ligations separating the balloon into three distinct balloons; *A*, *B*, *C*, balloons formed by the ligations at *K*, each balloon is independent of the other; *X*, small wire coil springs extending from the balloon through the rubber tube to *Y*. *1*, *2*, *3*, rubber tubes leading from balloons to manometers.

At this time (January, 1927) we took up the work. Normal contractions of the descending colon of several guinea pigs were recorded over a period of several weeks.

In order to study the character of the colon contractions a system of three balloons was devised. These tubes were so arranged that a single balloon could be drawn over them and tied off at three places to form the three independent balloons. In order to prevent collapsing of the tubes and balloons and to give rigidity to the system, small wire coil springs were inserted into the tubes as shown in Fig. 1. The tubes were fastened together in the form of a triangle with rubber cement and mastic. The springs protrude about one inch from each rubber tube preventing collapse of the first balloon and keeping the other two balloons from sticking to the rubber tubing. They also enable one to tie the balloons without constricting the rubber tubing.

This method of study shows the character of contractions in the descending colon of guinea pigs to be peristaltic, mass, and probably pendular or segmentation, i.e., any one balloon initiating a contraction wave in either direction.

*From the Physiological Laboratory of the University of Chicago, Ill.
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The normal activity shows considerable variation during a single test and also from day to day. In anaphylactic shock recognizable by the gross manifestations, the contractions of the descending colon are augmented but not sufficiently so to make this a satisfactory method for recognizing anaphylaxis.

A small balloon inserted in the vagina of a virgin pig under urethane anesthesia shows marked variations in the motor activity. Whether these variations are dependent upon the position of the balloon, the manometer pressure, the depth of anesthesia, or the estrus cycle, has not been determined. Considerable augmentation of the contractions is caused by histamine, but the normal variations are too great for using this as a method of recognizing mild anaphylactic shock.

The established uterine strip method and our partial success with the vagina led us to attempt methods of recording uterine activity in vivo without anesthesia. A Thiry-Vella fistula (dog) was prepared, and one horn of the uterus was wrapped around the intestinal segment. After healing a balloon was inserted into the fistula so as to lie directly under the uterine loop, but the intestinal contractions are so great normally that the recognition on the tracing of uterine movements is practically impossible. However, when oxytocin* is injected contractions of the intestinal loop are almost completely stopped for a few minutes, and the tone is raised, Fig. 2-*A*. This rise in tone is probably due to uterine contractions, since it does not appear in fistulas not in contact with a uterine loop (Fig. 2-*B*). The paralysis of intestinal movements by oxytocin is very temporary, and when the contractions return, further study of uterine activity is difficult.

Twenty days after sensitization the injection of 1.5 c.c. of fresh egg white into a dog with a utero-Thiry-Vella fistula caused a slow rise in tone, beginning ten minutes after the injection, Fig. 2-*C*. About forty minutes after this anaphylactic injection oxytocin was injected. The intestinal contractions were stopped, but the change in tone was very slight. The rise in tone following the injection of egg white was probably due to anaphylaxis. The failure of oxytocin to produce the typical tone change was probably due to the anaphylactic contraction of the uterus. While oxytocin diminished the tone of the intestine, as it does normally, there was no sharp increase in tone due to uterine contraction. This is probably evidence that the uterus was already contracted to a maximum by the protein.

The work of Weitz and Vollers[1] on pregnant women, in which they were able to record rhythmic activity of the uterus, suggests that records of uterine action might be made from the uteri of dogs if lifted and sewed to the ventral peritoneum. An operation was performed in which the horns of the uterus were sewed to the peritoneum on each side of the midline. In one case an abdominal muscle was removed so as to produce a hernia in which the uterus could lie. Attempts were made to record uterine movements from this preparation by means of a frog lever attached to a straw which rested over the position of the uterus. Intestinal movements interfered to the extent that it was not possible to differentiate them from those due to uterine action.

In March, 1928, an operation was attempted, the idea of which had sug-

*The oxytocin used in this experiment was furnished by the Parke Davis Laboratories.

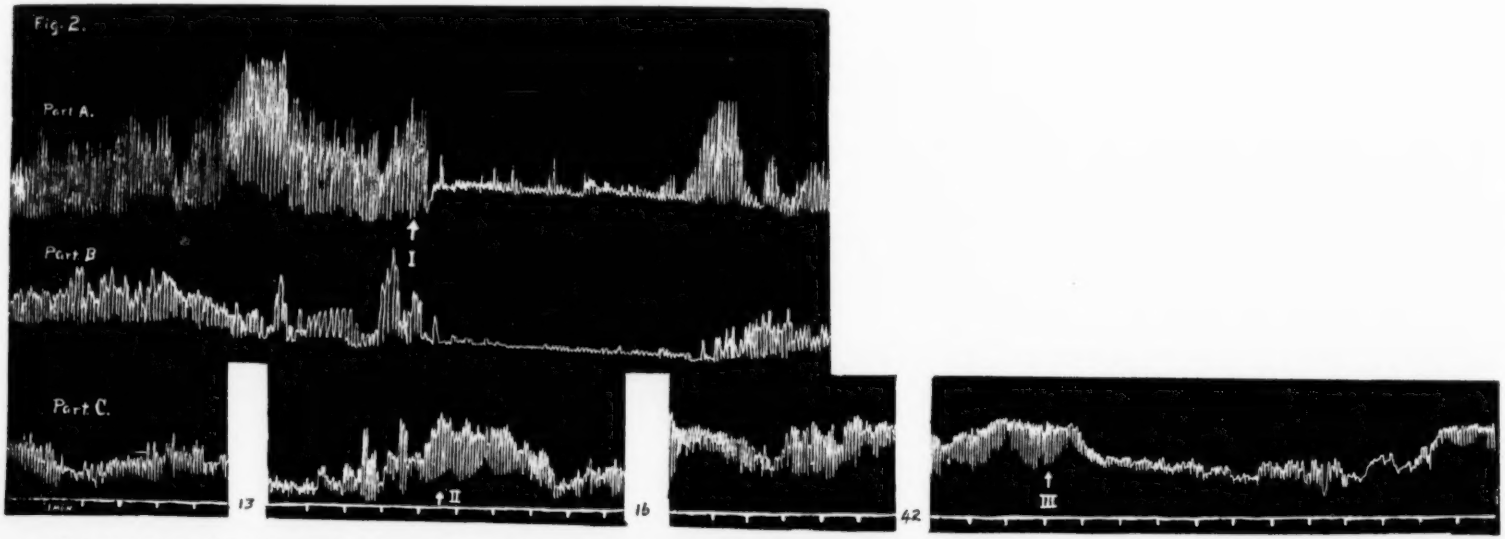

Fig. 2.—*A*, *B*, simultaneous balloon tracing from a double Thiry-Vella fistula (dog); I, injection of 0.5 c.c. of oxytocin. *A*, the uterine horns are wrapped around the intestinal loop; *B*, intestinal loop used for control; *C*, balloon tracing from Thiry-Vella fistula around which the uterine horns are wrapped. II, injection of 1.5 c.c. egg white (anaphylactic injection). III, injection of 0.5 c.c. of oxytocin.

Fig. 3.—*A*, *B*, simultaneous tracing from the vagina and uterine exposure; 1, injection of 0.5 c.c. oxytocin. *A*, balloon tracing from vagina, within two inches of uterine bifurcation; *B*, plethysmograph tracing from uterine exposure.

gested itself early in the work but which had been postponed in order to try what seemed to be simpler surgery, the idea being to bring the uterus entirely outside the body for study.

A loop of the uterus about 1.5 inches long, the point of bifurcation, and about one-half inch of the vagina were brought entirely outside the body. The blood vessels running parallel with the uterus were also brought out. The peritoneum, muscle and skin were closed underneath, care being taken not to compress the blood vessels. The surgery is very simple and the animal recovers promptly. Such a preparation is serviceable for experiments for several months, and probably indifinitely.

It was possible to place a small plethysmograph around the exposed uterine loop and record the spontaneous movements of the intact organ. A normal record from this uterine preparation showed pulse, respiration and spontaneous uterine movements. In our preparation, contractions caused a buckling of the exposed loop raising the water level in the manometer. A balloon inserted through the vulva, to within two inches of the uterine bifurcation, showed spontaneous activity, but not apparently correlated with the uterine movements.

The injection (intravenous) of oxytocin (Fig. 3) caused partial tetany of the uterus within ten seconds, while a contraction of the vagina two inches from the uterine bifurcation was not elicited until one minute after the injection. The effect of oxytocin on the uterus can be clearly seen for at least one hour. The pulse, which is obliterated by the partial tetany, begins to return in about fifteen minutes. The duration of the vaginal contractions is only about eight minutes.

A subcutaneous injection of 0.06 mg. of ergotamine caused a partial tetany of the uterus within five minutes. This tetanic condition lasted about eight minutes, followed by augmented contractions for several hours. At the onset of tetany the pulse disappeared and did not return for an hour or more. Oxytocin did not give a characteristic effect until forty-eight hours had elapsed following the ergotamine injection when normal activity was restored.

SUMMARY

1. Contractions of the descending colon in guinea pigs, studied by the triple balloon method, are augmented during anaphylaxis only when the shock is pronounced.

2. Normal variations in the activity of the descending colon and vagina of guinea pigs are opposed to their use as indicators of anaphylaxis.

3. Graphic records of uterine activity can be obtained from the exposed uterus of dogs and the animal kept in good condition several months (probably indefinitely).

4. The exposed uterus of the unanesthetized animal offers good opportunity for study of the uterus in situ.

We are indebted to Dr. A. J. Carlson, under whose directions this work was conducted, and wish to thank Dr. C. S. Smith and Dr. O. H. Horrall whose surgical assistance was invaluable.

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THE SEDIMENTATION VELOCITY OF ERYTHROCYTES IN THYROTOXICOSIS*

By J. M. Mora, M.D.,† and J. T. Gault, M.D.,‡ Chicago, Ill.

IT IS somewhat more than a decade since Robin Fahraeus[1] first reported his observations on the sedimentation velocity of red blood cells. The widespread interest aroused by this apparently simple yet extremely complex phenomenon was evidenced by the remarkably voluminous literature that accumulated in this brief period. The original observations dealt with the study of the reaction in pregnancy, but it has since been carefully studied in a great variety of conditions.

The sedimentation speed of the red corpuscles in man is subject to physiologic variations according to age, sex, and, in women, to the absence or presence of pregnancy. (The latter is the only physiologic condition in which the sinking velocity is increased.) The lowest figures are obtained in the newborn; in adults, men generally show considerably lower figures than women. During gestation, the increased sedimentation velocity becomes apparent in the second month, reaches its highest figures at term, then rapidly recedes during the puerperium. It is more or less increased in all acute general infections and appears to be of considerable value as a source of information as to the occurrence of complications. In chronic infectious diseases, such as syphilis and tuberculosis, the sedimentation speed is quite markedly increased. The field of internal medicine in which this reaction has been most fully investigated and the most important results obtained is pulmonary tuberculosis.

As first noted by Westergren[2] and since corroborated by other investigators, the sedimentation speed may reflect better than the body temperature the intensity of the tuberculous process. Gynecologists have found the reaction of value in differentiating pelvic inflammatory lesions from neoplastic and other lesions. To quote Fahraeus:[3] "It may be said that a reduction in the suspension stability of the blood is one of the most common general reactions of the organism in disease, perhaps the most common. In this respect it may best be compared with such reactions as pyrexia and leucocytosis. . . . . On account of its completely nonspecific character, the reaction has, as a rule, proved less instructive in respect to the diagnosis of the disease, than as regards the activity or intensity of the morbid process."

The hydrodynamics of the sedimentation reaction are very complex. According to Fahraeus[3] the factors which influence the sinking velocity are: the concentration of the suspension (i.e., the relative number of corpuscles), the radius of the particle, and the tendency of the red blood corpuscles to form

*From the Surgical Service of Dr. H. M. Richter, Mt. Sinai Hospital, Chicago, Ill.
Received for publication, September 19, 1929.
†Instructor in Surgery, University of Illinois College of Medicine.
‡Resident Physician, Mt. Sinai Hospital, Chicago, Ill.

aggregations or rouleaux. The latter is said to determine the sedimentation speed. Increased rouleaux formation is caused mainly by increase in the serum globulin and fibrinogen. The lipoids of the plasma have also been considered of importance,[2, 4] as have the gases of the blood. Experiments in vitro by Leendertz[5] have shown that the sinking velocity is increased if oxygen is bubbled through citrated blood, whereas carbon dioxide decreases it.

The most commonly used methods of determination are those of Fahraeus[1] and Westergren[2] (the "distance method"), in which the sedimentation speed is obtained by measuring the distance passed by the uppermost layer of red cells from the upper meniscus of the fluid column in a given time, and that of Linzenmeier[6] (the "time method"), in which the time in minutes is taken that is required for the corpuscles to sink a certain distance marked on a specially calibrated tube. Using the latter method, the normal time has been found to average 850 to 1000 minutes for women, and 1000 to 1200 minutes for men (Linzenmeier,[6] Friedlander,[7] Löhr.[8]) Briefly, the technic consists of mixing 0.2 c.c. of freshly prepared 5 per cent sodium citrate solution, with 0.8 c.c. of blood, and noting the time required for the cells to sink 18 mm.

The influence of thyrotoxicosis on the sedimentation velocity seems to have been studied but little. Uyeno,[9] in 1926, studied 19 cases. In 8 the rate was markedly increased, in 6 slightly increased, in 3 the rate was normal, and in 2 cases it was decreased. DeCourcy[10] reported 7 cases of mild hyperthyroidism in which the sedimentation rate was increased. Tschernosatonskaya[11] recently studied 42 cases, and without exception the rate was increased. He believes that the sedimentation velocity parallels the severity of the disease process.

We studied 30 cases of thyrotoxicosis before and after operation. (A control series of 20 other surgical cases was also included, these consisting of 11 inguinal herniotomies, 3 ventral herniotomies, 2 hysterectomies, and 4 thyroidectomies for nontoxic adenomatous goiter). In 11 of the 30 cases, we were able to note the effect of the administration of iodine. All of the 30 cases showed a definite increase in the sedimentation velocity before operation, but the operative relief of the hyperthyroidism did not uniformly affect the sedimentation speed. In 6 of the 11 cases (in whom the rates were taken before and after the administration of iodine) there was a decreased sedimentation speed after the period of iodinization, coincident with a drop in the basal metabolic rate. The other 5 cases showed an increased rate during the same period. After maximal thyroidectomy there was a decrease in the sedimentation speed in 14 of the 30 cases; in 13 of the cases there was an increased rate and in the 3 remaining cases the rate remained about the same as before operation. It was rather confusing to note the increased rate after operation in nearly one-half of the cases, particularly since 8 of these patients were definitely hypothyroid. We are at a loss to explain this phenomenon. There appeared to be little parallelism between the sedimentation speed and the basal metabolic rate. It will be observed that all the postoperative velocities were rather rapid, and as these cases were studied from one to six months following thyroidectomy, it may be that these patients require a much longer time to

regain their normal state than we ordinarily suppose. On the basis of this study it appears that the sedimentation rate is of little clinical value in thyrotoxicosis.

SUMMARY

The sedimentation speed of erythrocytes was studied in 30 cases of thyrotoxicosis, by the Linzenmeier method, before and after thyroidectomy. The rate was increased in all cases before operation. The administration of iodine decreased the speed of sedimentation in 6 out of 11 cases, and increased it in 5 cases. Thyroidectomy was followed by an increased rate in 13 cases, and by a decreased speed in 14 cases. There seemed to be little parallelism between the sedimentation velocity, the basal metabolic rate, and the clinical picture.

We desire to acknowledge our indebtedness to Mrs. V. Haste and Mrs. B. Rieger, for their aid in this work.

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A TECHNIC FOR OPERATIONS ON SEVERAL SMALL ANIMALS AT ONE TIME BY USE OF AMYTAL ANESTHESIA*

By Robert L. Johnston, M.D., Cleveland, Ohio

THE technic herein described enables one to carry on without the assistance of an anesthetist three to five aseptic surgical operations on small animals such as rats, guinea pigs, or rabbits, with a single sterile preparation. By this technic as many as ten animals can be operated upon within a single morning.

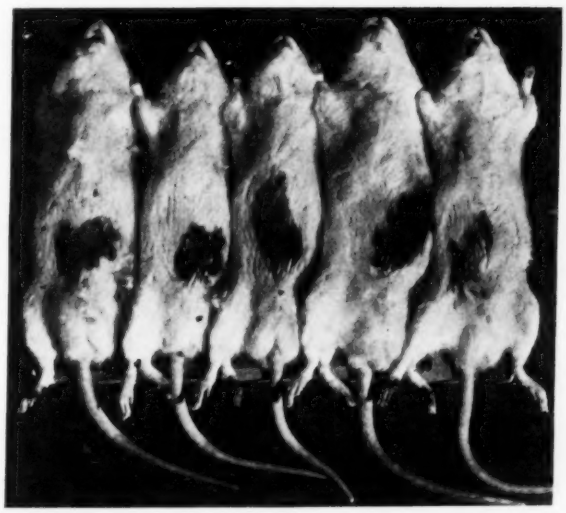

Fig. 1.—Simultaneous operation on five rats under amytal anesthesia.
Stage 1. Local application of iodine at site of injection of amytal. Note attachment of animals to operating board by means of adhesive over legs.

ANESTHETIC

Amytal (Lilly) is purchased in 50 gm. quantities and prepared according to the directions of the manufacturer, namely, to one gram of amytal 8.85 c.c. of N/2 sodium hydroxide solution a is added with stirring; the mixture is placed over a boiling water-bath and stirred frequently. Solution occurs within from ten to twenty minutes with the formation of amytal-sodium, or sodium-iso-amyl-ethyl-barbiturate. When dissolved and made up to a 10 per cent solution, it is ready for use on large animals. For use on small animals, however, it should be further diluted to a 1 per cent solution, otherwise the danger of overdosage is increased tenfold. Only fresh solutions should be used; clouded solutions should be discarded.

*From the Cleveland Clinic, Cleveland, Ohio.
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TECHNIC

The animals are weighed and the appropriate amount of the 1 per cent solution calculated for each animal. With a fresh stock of amytal, 0.060 gm. per kilogram is adequate to maintain complete surgical anesthesia for two or more hours. Rabbits often require 0.075 gm. per kilogram, sometimes more.

An assistant holds the animal while a 2 per cent solution of iodine is applied to the abdomen at the site chosen for injection. The computed dose is given intraperitoneally from a Luer syringe, using a small hypodermic needle. Usually by the time the last of five animals is injected (four or five minutes) the first animal is ready to be attached to the operating board which consists of a piece of wood board about 7 by 10 inches in size. The animal's legs can be quickly attached by means of small strips of adhesive tape, overlapping in the manner shown in Fig. 1. By the time all the animals are attached to the board, surgical anesthesia should be complete in all.

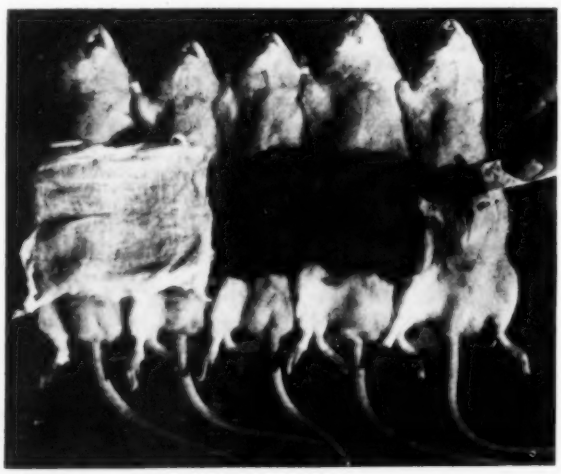

Fig. 2.—Simultaneous operation on five rats under amytal anesthesia.
Stage 2. (Right) Application of barium sulphide to abdomen.
Stage 3. (Left) Warm, wet cloth applied over barium sulphide.

The field of operation is next sprinkled liberally with barium sulphide and then covered with warm, wet cloths to effect saturation with water, as shown in Fig. 2. Within five minutes, the barium sulphide can be swabbed off with wet gauze, bringing with it all the underlying hair (Fig. 3). The skin is then dried with gauze and is painted with the usual alcoholic solution of iodine. The operator scrubs, puts on a sterile gown, and drapes all the animals at one time, after placing pieces of gauze between them (Fig. 4). The desired operative procedures are now carried out (Fig. 5), and the closures made, peritoneum with muscles, then the skin, by means of silk sutures. We have found the continuous, hidden, subcuticular skin suture very satisfactory for work on rats and rabbits. Occasionally, however, we use interrupted sutures. Usually the skin sutures are extruded, but those in the muscular layer are rarely extruded, as shown by subsequent autopsy.

Occasionally, an overdose is given, as a result of struggling by the animal

while the injection of the anesthetic is being made. Also, sometimes when operative procedures are prolonged, one or more of the rats will begin to recover, so that a second injection is required, with resultant overdosage. For these reasons, when animals are operated upon in such numbers, there is a mortality of from 10 to 20 per cent. This mortality, however, is negligible when we take into consideration the time saved by the use of a method which

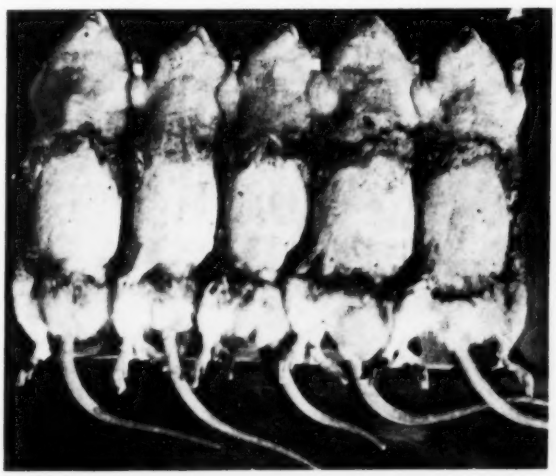

Fig. 3.—Simultaneous operation on five rats under amytal anesthesia. Stage 4. Field of operation after removal of barium sulphide.

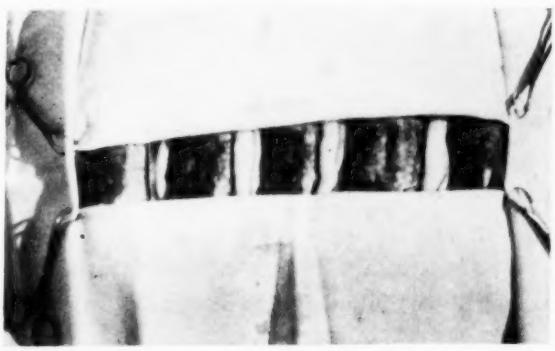

Fig. 4.—Simultaneous operation on five rats under amytal anesthesia. Stage 5. Iodine applied to field of operation and drapes applied.

makes it possible for one operator to perform as many as twenty operations in a single day without an anesthetist.

Although this paper is not intended to be a study of amytal anesthesia, but rather a description of its uses in connection with experiments on small animals, nevertheless it may be well to draw attention to the works of the following authors as examples of the direct study of this substance as an anesthetic:

Page and Coryllos[1] described a method of preparation of amytal solutions

using ethylene glycol as the solvent and preservative. In their experience, a dosage of 45 to 50 mg. per kilogram is most suitable for intravenous administration in dogs. They employ a dosage 15 to 20 mg. higher for intraperitoneal administration as advocated by Edwards and Page.[2]

Swanson and Page[3] state that the intravenous minimal effective dose for amytal in cats is 45 mg. per kilogram as compared with 155 mg. per kilogram for barbital. They find the induction speed an hour shorter with amytal than with barbital. They give statistics of their own work, together with statistics given by other authors, which show the minimal effective dose as compared with the minimal fatal dose in over 500 animals of various species.

In a series of ten experiments on rabbits, Underhill and Sprunt[4] found that eight gave evidence of distinct hyperglycemia following doses ranging from 50 to 100 mg. per kilogram, whether the amytal was given by mouth,

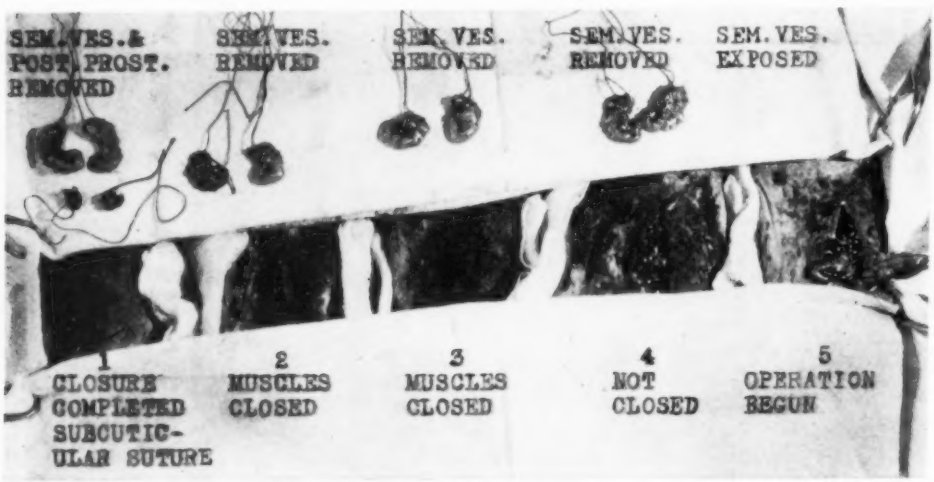

Fig. 5.—Simultaneous operation on five rats under amytal anesthesia. Illustration of method of closure.

subcutaneously, or intraperitoneally. They call attention to the fact that Weiss[5] has shown that amytal produces hyperglycemia in the cat and the dog.

Hines, Boyd, and Leese[6] found that dogs under amytal anesthesia have a decreased capacity to assimilate injected glucose.

Hines, Leese, and Barer[7] compared the glycogen content of liver and muscle in normal animals with that in animals anesthetized with amytal, using eight dogs. Three hours following the injection of glucose solution, they found the glycogen content of muscle to be the same as prior to amytal anesthesia. They found, however, that the liver glycogen was increased to double the amount in the unanesthetized animals.

Investigators using amytal anesthesia on dogs are familiar with a characteristic shivering which occurs when the anesthesia begins to diminish. I have noticed this phenomenon commonly in dogs, but never in rats or rabbits. It has been noted that these smaller animals, which do not shiver under amytal anesthesia, very readily lose their body heat so that in prolonged operative

procedures the superficial tissues actually feel definitely chilly to the touch. It is believed that postoperative complications are reduced materially by artificially combating this heat loss. The mechanism of this heat loss is apparently as follows: carbohydrate metabolism is suppressed, as evidenced by the existence of hyperglycemia. Carbohydrate formation or glycogenesis is not suppressed as much as the glucose utilization, as evidenced by the increased glycogen content of the livers of animals anesthetized with amytal as compared with the livers of unanesthetized animals. It follows that suppression of glucose utilization lowers the heat production which is combustion of both carbohydrates and fats. In dogs, shivering combats this heat loss. In animals which do not shiver and which are smaller, the heat loss is greater, due to the absence of shivering, and also to the larger relative body surface for heat radiation. Uncombated heat loss subjects the animals to postoperative complications, especially to pulmonary complications.

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NOTE ON THE DETERMINATION OF INORGANIC PHOSPHATE OF SERUM AND SPINAL FLUID ON THE SUPERNATANT FLUID FROM CALCIUM ESTIMATION*

By Clare Lowenberg, B.S., and Marjorie R. Mattice, A.B., New York, N. Y.

IT IS often difficult to obtain sufficient blood serum or spinal fluid for determination of both calcium and inorganic phosphate in very young children. Gunther and Greenberg[1] recently published a modification of the Fiske and Subbarow[2] inorganic phosphate determination applicable to the supernatant fluid from the Clark-Collip modification of the Kramer-Tisdall[3] calcium determination. Following their suggestions we have determined inorganic phosphate by the Benedict and Theis[4] method on the supernatant fluid from the calcium determination.

PROCEDURE

In a 15 c.c. conical centrifuge tube place 2 c.c. serum, 2 c.c. distilled water, and 1 c.c. of 4 per cent ammonium oxalate. Mix thoroughly by gentle shaking. After thirty minutes, centrifuge. Decant the supernatant fluid into a wide-mouth tube and invert the centrifuge tube to drain, proceeding as usual with the calcium determination.

To 4 c.c. of the supernatant fluid add 2 c.c. of water and 4 c.c. of 20 per cent trichloracetic acid. Mix thoroughly and filter after ten to twenty minutes. Measure 5 c.c. of the clear filtrate into a Myers' sugar tube, add 1 c.c. water, 2 c.c. 0.1 N potassium permanganate, and without delay 1 c.c. molybdic sulphuric acid reagent and 1 c.c. bisulphite hydroquinone reagent. Prepare the standard as usual. Mix, stopper loosely, and heat in a boiling water-bath for ten minutes. Cool and compare in the colorimeter, setting the standard at 15.

Calculation.—

$$\frac{15}{R} \times 0.025 \times \frac{100}{0.8} = \text{mg. P / 100 c.c. serum.}$$

The 4 c.c. supernatant fluid used represent 1.6 c.c. serum. Five c.c. of the trichloracetic acid filtrate therefore represent 0.8 c.c. serum. The strength of standard used is 0.025 mg. phosphorus in 3 c.c.

The procedure for estimation of inorganic phosphate in spinal fluid on the supernatant fluid from the calcium is similar to that in serum, except for a change in proportions made necessary by the smaller amount of inorganic phosphate present in spinal fluid.

Add 1 c.c. of ammonium oxalate and 1 c.c. of water to 2 c.c. of spinal

*Department of Laboratories, New York Post-Graduate Medical School and Hospital.
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fluid. Mix 3 c.c. of the supernatant fluid and 2 c.c. of 20 per cent trichloracetic acid. After ten minutes filter until clear through a small filter paper (not over 5.5 cm.). Take 4 c.c. of the filtrate, 3 c.c. of 0.1 N permanganate, and 1 c.c. each of water, molybdic acid and hydroquinone reagents for the color development.

Calculation.—

$$\frac{15}{R} \times 0.025 \times \frac{100}{1.2} = \text{mg. P / 100 c.c. spinal fluid.}$$

DISCUSSION

In Table I are shown the results obtained by determination of inorganic phosphate on serum and on supernatant fluid from calcium determination. The variation is negligible in practically every case.

TABLE I

SPECIMEN	INORGANIC PHOSPHORUS OF SERUM MG./100 C.C.	INORGANIC PHOSPHORUS OF CALCIUM SUPERNATANT FLUID MG./100 C.C.	DEVIATION FROM SERUM VALUE MG./100 C.C.
1	4.1	4.0	−0.1
2	3.9	4.0	+0.1
3	3.9	3.9	0
4	4.6	4.8	+0.2
5	3.3	3.3	0
6	3.4	3.4	0
7	4.0	4.0	0
8	6.0	5.8	−0.2
9	2.1	2.2	+0.1
10	3.9	3.9	0
11	4.8	4.6	−0.2
12	3.6	3.7	+0.1
13	5.9	5.8	−0.1
14	3.1	3.3	+0.2
15	3.4	3.5	+0.1
16A	6.7	6.3	−0.4
16B	7.0	7.2	+0.2

In Table II are shown the results obtained by determination of inorganic phosphate of spinal fluid and in supernatant fluid from calcium determination.

TABLE II

SPECIMEN	INORGANIC PHOSPHORUS OF SPINAL FLUID MG./100 C.C.	INORGANIC PHOSPHORUS OF CALCIUM SUPERNATANT FLUID MG./100 C.C.
1	1.4	1.5
2	1.5	1.5
3	1.3	1.3
4	1.5	1.4
5	1.6	1.7
6	1.6	1.6
7	1.6	1.6
8	1.8	1.8
9	1.6	1.6

Determination of inorganic phosphate on the supernatant fluid from the calcium determination without the addition of permanganate (Table III) gave low results, the error increasing with larger amounts of phosphate.

TABLE III

SPECIMEN	INORGANIC PHOSPHORUS OF SERUM MG./100 C.C.	INORGANIC PHOSPHORUS OF CALCIUM SUPERNATANT FLUID MG./100 C.C.	DEVIATION FROM SERUM VALUE MG./100 C.C.
L	2.9	2.5	−0.4
M	5.2	3.6	−1.6
J	8.0	4.9	−3.1

Briggs[5] states that oxalates and citrates in such amounts as are used to prevent clotting of blood do not interfere with the acid development of the blue color, and thus plasma may be used instead of serum for the determination of inorganic phosphate. The two c.c. of 0.1 N permanganate added is nearly the theoretical amount required to oxidize the oxalate present.

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GASTROENTEROLOGIC TABLE WITH A SPECIAL DRAINAGE OUTFIT*

By Moses Einhorn, M.D., New York

WHILE engaged in the practice of gastroenterology, I have been in a position to observe closely the various types of tables used in this work. My observations point to the fact that there is at present no standard table, physicians using whatever is nearest at hand, such as a surgical table, a house couch, or any other common type. Each of these has its disadvantage, some being too high or too low for the convenience of the examiner; others, too soft or too hard for the patient's comfort. None of the tables mentioned allows for an adjustable headrest.

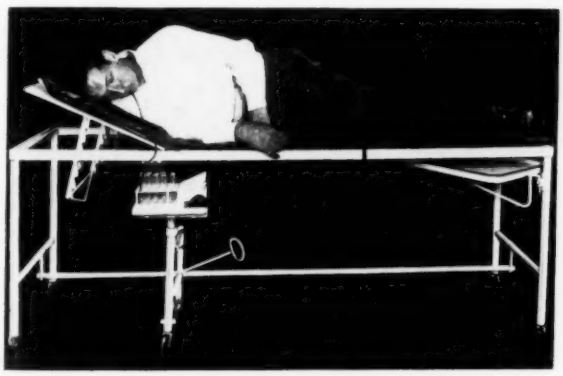

Fig. 1.

In using the tables for drainage work, a separate chair, placed on a lower level than the table, is necessary for the arrangement of the test tubes. Any movement on the part of the patient may thus spill the contents of the tubes, and disturb the work of the drainage apparatus. In doing rectal work, an additional side table is necessary for the instruments.

Noting the disadvantages, and realizing the need of a standard table, I constructed a special table (Fig. 1) in order to eliminate these difficulties. The table is six feet long, two feet wide, and two feet, two inches high; and is constructed with a metal top, supported by four legs. It is covered with a soft comfortable leather pad, and has an adjustable head support. The table is reinforced in the middle by a longitudinal bar, to which is attached a special drainage outfit. The upper section of this outfit is composed of three parts; a special test tube rack with perforations to hold the tubes securely in place; a small box to contain the drainage supplies, and a concave support for the syringe. The whole is supported by an iron bar, which is telescoped in a

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tube, at the base of which is a large wheel. Extending from this tube, and perpendicular to it, are two parallel rods, also telescoped in a U-shaped iron tube, with a small wheel at its base. This U-shaped tube connects the entire drainage outfit to the longitudinal bar which reinforces the table. Also, there is a handle convenient for sliding it into the required position. Several small screws are attached to the outfit to allow for the adjustment thereof.

For rectal work, there is a special tray to hold the necessary instruments and supplies, which can be attached either to the right or left side of the table, at the convenience of the examiner.

In doing drainage work, the patient is placed on the table on his right side in a reclining position, with the right leg and knee extended, and the left leg and knee flexed, overlapping the right, thus bringing the duodenum lower than the stomach. The drainage outfit is then withdrawn from beneath the table by releasing the screw which holds it in place. The rack is released by another screw, and is drawn upward and the syringe supporter turned outward. As this whole moves up and down and in and out, it can be raised to and fixed in the required position. The test tubes are then placed in the perforated section of the rack, the syringe in the concave support, and the preparations are complete for the examiner to commence his work.

When the drainage work is over, the rack can be lowered, the syringe supporter turned inward, the screws refastened, and the entire outfit slid beneath the table.

For rectal work, the special tray is withdrawn, and the instruments and supplies placed thereon, within convenient reach of the physician. The work completed, the instruments are removed and the tray slid beneath the table.

ADVANTAGES

1. It is a standard table, of dimensions convenient for the examiner.
2. The soft cushioned leather pad adds to the comfort of the patient.
3. The adjustable headrest permits the head to be raised or lowered as required.
4. A complete drainage outfit is attached, and can be arranged in any position desired. This outfit includes the following:
   a. a test tube rack to hold the test tubes securely in place to avoid spilling.
   b. a small box to contain the supplies.
   c. a concave support that holds the syringe.
5. The rectal tray, for the necessary instruments, can be hooked on either side of the table, at the convenience of the examiner.

983 Park Avenue.

AN IMPROVED STIMULUS SELECTOR*

By Richard Homer Fitch, M.A., Madison, Wis.

IN EXPERIMENTATION involving the stimulation of the cortex at variable frequencies and intensities, it became necessary to devise a stimulus selector of a type which could be shielded to prevent amplifier pick-up and which would give constant frequencies and intensities at the settings utilized. The rotary commutator type of stimulus selector was found to possess several disadvantages for these purposes. The brushes have a tendency to skip, and it is difficult to secure a constant resistance break on the commutator surface; and the variation in line voltages supplying the motor to the rotary selector results in inconstant frequencies and potentials. A type of stimulus selector incorporating an oscillating neon tube, as developed in England by Daly,[1] was set up in an attempt to overcome these difficulties. It was found that in this arrange-

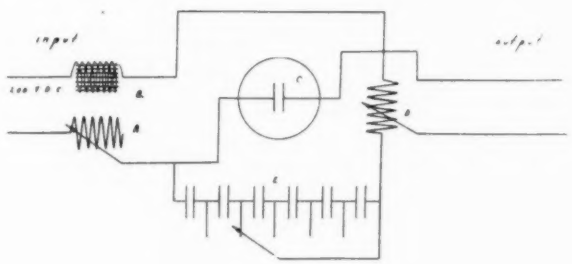

Fig. 1.—*A*, 0-5 megohm variable grid leak; *B*, Iron core choke (secondary of Thordarson audio transformer); *C*, Neon tube (supplied by Q.R.S. Company, Chicago); *D*, Output resistance, 50,000 ohms, variable; *E*, 0.5 M.F.D. condensers, insulated for 250 volts.

ment the voltages secured were too low, and the type of neon tube available on the market would not oscillate at the frequencies desired.

The set-up as devised is largely empiric, the different values having been arrived at through a more or less trial and error process. A neon tube was made up for us by the Q.R.S. Company of Chicago, which was found to be suitable. The commercial television tubes would not operate over a large range of frequencies, due to the fact that the electrodes are not equally spaced. This variation in the distance between them resulted in the entire electrode's surface not being involved in the oscillation. The tube furnished by the Q.R.S. Company possessed a constant intraelectrode distance and was found to operate satisfactorily at frequencies ranging from once in four seconds to one hundred per second. Higher frequencies can be obtained by use of a tube of smaller electrode area. If a very high potential output at high frequencies is desired, it is probably impractical to use this type of stimulus selector, as the potential varies inversely with the frequency.

*From the Department of Physiology, University of Wisconsin, Madison, Wis.
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The output circuit, as diagramed in Fig. 1, does not supply high enough voltages for stimulating all tissues, the maximum being approximately 15 volts. It can, however, be connected to the primary of a Harvard induction coil which, in our experience, gave a maximum potential of 85 volts.

Thinking that the use of the step-up transformer and an iron core choke coil, which was included in the D.C. supply main, might change the characteristics of the oscillations from those described by Pearson and Anson,[2] an attempt was made to record the form of the stimulating current by means of the string galvanometer. It was found that a string sufficiently tight to follow the rapid change would continue to vibrate at its own period after the discharge from the selector had ceased giving the impression of a polyphasic fluctuation with decrement. If the string was slackened to its critical aperiodic point to prevent its vibrating, the resulting form was monophasic but was unduly extended in time, the string being unable to follow such a rapid change.

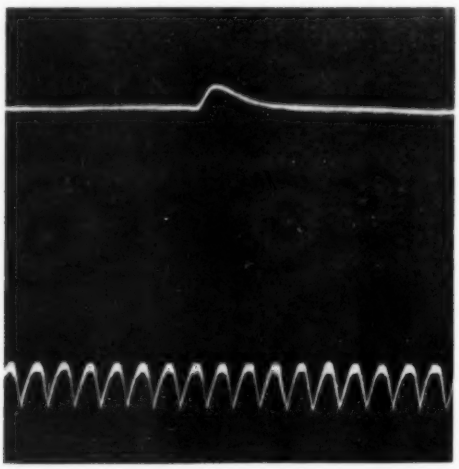

Fig. 2.—Oscillograph of wave form. *A*, Stimulus selector; *B*, Time 1/1000 second.

Oscillographic pictures were then taken (Fig. 2) by means of a Westinghouse oscillograph with a period of approximately 2,000 cycles. The period of rising potential was found to occupy 0.52 sigma, and the total duration of the impulse 2.6 sigma, the form being monophasic.

This form of stimulating current is of particular advantage, because the lower frequencies possess the same rate of potential change as the higher, and consequently have the same stimulating value.

The operation of the set-up simply involves the adjustment of *A* and *E* (Fig. 1) for varying the frequency, and setting *D* according to the potential output desired. The high voltage direct current was secured from a radio "B" eliminator but can also be obtained from "B" batteries or motor generator.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TISSUE: A Short Method of Clearing Plant Tissues, Simpson, J. L. Stain Tech. 4: 131, 1929.

The material to be studied is placed in lactic acid of a concentration of about 75 per cent. Whole blossoms or large parts such as the pistil may be immersed in the medium in an open watch glass. Sections of fresh material, cut fairly thick by hand, may be mounted in the acid on a slide and the cover glass applied. In either case the specimen is then placed in a constant temperature oven and kept at about 50° C. until clear. The time required varies with the material. Sections of floral parts, young fruits or succulent stems become clear in two or three hours. Whole parts or thick edges of such material have required, in the writer's experience, about twelve hours. It is often convenient to leave them overnight.

If permanent slides are desired, the lactic acid may be thickened by slow evaporation in the oven until it is almost hard. The edges of the preparation may then be sealed. For this purpose, a mixture of gum mastic and paraffin, applied with a hot iron wire, has been found suitable. As lactic acid is hygroscopic, the slides become very sticky if left unsealed. In cleaning up such slides it is useful to remember that lactic acid is soluble in alcohol.

CANCER: A Serum Test for the Diagnosis of Cancer Based On a New Theory of Etiology, Gruskin, B. Am. J. M. Sc. 177: 476, 1929.

Preparation of the amboceptor for carcinoma: Mammalian embryos are used (calves, sheep, pigs). They must not be in a later stage than the second month of pregnancy. This is readily recognized by their relative smallness, and by the smoothness of the skin (for instance, there is no formation of hair). In securing these embryos, the abdomen must be opened under sterile conditions. The pancreas and submaxillary glands are dissected out, and placed in a sterile dish. A hypotonic salt solution is poured over these, and if possible, allowed to freeze, the object being to permit an easier removal of the fibrous capsule. The capsule and the ducts, etc., are then removed by careful manipulation with small tissue forceps. The epithelial tissue is picked out. It is placed in a mortar in which sterile copper gauze is inserted (to facilitate the maceration) and macerated. Salt solution is added and thoroughly mixed and rubbed up with the macerated tissue until the fluid becomes milky or opalescent in appearance.

The cells suspended in salt solution are centrifuged until the supernatant salt solution is perfectly clear; the salt solution is then discarded and the epithelial cells are placed in a porcelain dish and dried at a temperature of 75° C. to a doughy consistency. During this period, it is essential that the tissue be stirred thoroughly at fifteen-minute intervals in order to permit uniform drying. The tissue is then placed in a glass-stoppered bottle to which is added three times its volume of acetone. The mixture is permitted to stand in the ice chest for twenty-four hours with frequent shaking. The acetone is then poured off and replaced by one and a half volume of fresh acetone for another twenty-four hours. The acetone is again decanted and the tissue is placed in a mortar to which five times its volume of absolute alcohol is added. This is macerated for about fifteen minutes until the alcohol becomes somewhat milky. The mixture is then kept in the ice box for five days during which time it is forcibly shaken at two-hour intervals. The alcohol extract is then decanted and is ready for use.

Antigen for Sarcoma. A mixture of Wharton's jelly and red bone marrow of calf embryo is macerated in a mortar to which two volumes of salt solution are added. This is placed in a porcelain dish at a temperature of 75° C. until the mixture becomes grayish in color. The salt solution is then decanted and the tissue is placed in three times its volume of acetone for twenty-four hours. The acetone is then decanted and replaced by one and a half volumes of acetone for twenty-four hours. The acetone is again decanted and the tissue is placed in a mortar and macerated with five times its volume of alcohol for fifteen minutes until the alcohol extract assumes a light-brown color. The mixture is then placed in a glass-stoppered bottle in the ice chest for five days, during which time it is shaken at two-hour intervals. The alcohol extract is then ready for use.

Technic of Test: Five to ten minims (depending upon titration) of antigen is placed in a test tube, to which 30 minims of suspected serum (which has been previously diluted 50 per cent with normal salt solution) is added slowly drop by drop. This is allowed to stand for two minutes. It is then agitated until flocculation takes place. Ten minims of salt solution are added and are allowed to stand for two minutes. It is agitated again and read against a frosted electric light. A positive reaction is indicated by flocculation. A negative reaction produces no flocculation. It is important that the drops be added slowly and at regular intervals. Adding the drops too rapidly might give a negative reaction in positive cases. It is also important to distinguish between air bubbles and true floccula. The former always rise to the surface, whereas the latter settle to the bottom.

If the suspected serum has a very low specific gravity and looks watery, it is best not to mix serum with salt solution, but use it in its original state, as adding salt solution is liable to render a positive serum negative.

Titration: A known positive and negative serum are employed in order to determine the least amount of antigen necessary to produce a positive reaction. When this end-point is reached, the same amount is used on the known negative and should give a negative reaction. It is essential to determine this end-point accurately and utilize the least amount of antigen necessary to produce a flocculation. If the known positive of this amount is exceeded by even a few minims, a flocculation might take place in the negative serum.

Precautions: The following precautions should be observed in the preparation of the material: It is essential that the capillary pipettes used in the test should be of equal caliber. It is evident that any difference in the caliber of the pipettes would render the test inaccurate. It is also very important that the cells for the antigen and amboceptor be prepared of pure embryonic cells of the type desired, for example, the capsules and ducts of pancreas and submaxillary gland must be removed thoroughly so that no remnants of fibrous tissue will be left. In the preparation of the connective tissue amboceptor as well as the antigen, Wharton's jelly must be free from blood. The antigen must be dried until it assumes a doughy character, not more nor less than that. Special care should be taken to avoid scorching the tissue while drying. It is essential that the drying process be continuous, proceeding to the finish without stopping.

SPINAL FLUID: Microchemical Determination of Protein, Samson, K. Biochem. Ztschr. **208**: 262, 1929.

In special centrifuge tubes, having a portion of 0.08 c.c. capacity calibrated in 0.002 c.c., are placed 0.6 c.c. of spinal fluid and 0.3 c.c. Esbach's reagent and 0.6 c.c. spinal fluid and 0.6 c.c. saturated ammonium sulphate. The solutions are thoroughly mixed and left standing exactly thirty minutes and two hours respectively. They are then centrifuged at a speed of 3000 revolutions per minute for exactly forty and twenty minutes respectively. The value obtained in the first tube represents the total protein content. In the second tube the supernatant fluid is removed with a fine pipette, the precipitate of globulin is dissolved in 0.6 c.c. of H_2O, then precipitated with 0.3 c.c. Esbach's reagent. After standing exactly thirty minutes it is centrifuged exactly forty minutes. This gives the value for globulin, the albumin being the difference between these 2 results.

BLOOD CHEMISTRY: Tungstomolybdic Acid as a Precipitant for Blood Proteins, Benedict, S. L., and Newton, E. B. J. Biol. Chem. 83: 357, 1929.

A protein precipitant is described which does not cause the loss of any nonprotein constituents and which thus permits the quantitative recovery of thionine and glutathione from blood. It is prepared and used as follows: 10 gm. of pure ammonia-free molybdic acid is treated in a flask with 50 c.c. of N NaOH and the mixture boiled for one to two minutes. A practically clear solution should result. About 150 c.c. of H_2O is added and the cooled solution is mixed with a solution of 80 gm. sodium tungstate dissolved in about 600 c.c. H_2O. This mixed solution is diluted to 1 liter. The acid employed during the precipitation is 0.62 N sulphuric acid prepared by diluting 620 c.c. of N acid to 1 liter. The precipitation of blood proteins is carried out as in the familiar tungstic acid precipitation.

ACETOACETIC ACID IN URINE: Simple Determination of Melka, J. and Klein, F. Bratislav. Lekarske Listy 8: 188, 1928.

The acetoacetic acid in 5 c.c. urine is decomposed by the addition of 12 drops of N sulphuric acid and heating in a water-bath for fifteen minutes. After cooling and making alkaline with NaOH, ferric chloride solution is added and sufficient 1 per cent solution of acetoacetic acid to give a color reaction of the same intensity as that obtained from the original sample of urine. The amount of acetoacetic acid added is equal to that originally present.

TYPHOID: A New Enrichment Method for Typhoid and Paratyphoid Bacilli In Water, Hader, F. Zentr. Bakt. Parasit. 113: 353, 1929.

A stock solution of malachite green of 1:250 and of brilliant green 1:200 was used. Mix 38.5 c.c. of sterile water, 5 c.c. of sterile bile and 5 c.c. of bouillon, and add the material to be examined. Add 0.8 c.c. of stock malachite and 0.1 c.c. of brilliant green, drop by drop. Incubate three hours and add 0.45 c.c. of malachite and 0.15 c.c. of brilliant green and continue the incubation. The modification is very satisfactory.

VACCINES: A New Method of Producing Bacterial Vaccines, Vignati, J. Zentr. Bakt. Parasit. 113: 71, 1929.

Bacterial suspensions were made in 5 to 10 per cent $CuSO_4$ solutions allowed to stand at room temperature for four to six hours, centrifuged and diluted with the following solution: $Na_2S_2O_3.5H_2O$ 25 gm., Na_2SO_3 2.5 gm., distilled water 1000 c.c. These vaccines were just as antigenic and less toxic than heat-killed cells.

PNEUMONIA: Icterus Index Studies In Lobar Pneumonia, Elton, N. W. N. Eng. J. M. 201: 611, 1929.

The following conclusions are advanced:

1. In primary lobar pneumonia a latent jaundice constantly occurs.

2. The icterus index is often found in the range of clinical jaundice, yet clinical jaundice may not definitely be present.

3. The icterus index reaches its highest values in cases terminating in true crisis. No fatal case in this series had an index exceeding 17.

4. The latent jaundice usually subsides precipitously upon the establishment of a fluid pleural exudate.

5. The graph of the daily icterus index curve of the cases in this series shows a rising curve, occurring in the presence of a purely intraalveolar pneumonic process until crisis or death, and a declining curve in the presence of a fluid pleural exudate. The latter type of curve was always associated with cases that were potential empyemas.

6. Conclusions based on etiology must be deferred. Although the pneumococcus, which is bile-soluble, is commonly regarded as the etiologic agent in primary lobar pneumonia, it cannot be stated that it was the causative organism in all the cases of this series which underwent the phenomenon of crisis.

CHOLECYSTITIS: Bacteriological Study of a Group of Diseased Gall Bladders: Branch, C. F. N. Eng. J. M. 201: 308, 1929.

1. In chronic cholecystitis the gall bladder is infected in approximately 12 per cent of the cases.

2. In acute cholecystitis the gall bladder is infected in approximately 75 per cent of the cases.

3. Approximately 40 per cent of all gallstones are infected.

4. Bile in which the bile salts have a normal or high concentration has a marked inhibitory effect on bacterial growth.

5. The predominating organisms recovered from infected gall bladders in the order of frequency are B. coli, streptococci and staphylococci.

6. The organism recovered from a case of acute cholecystitis is not necessarily the primary etiologic factor responsible for the development of the acute lesion.

7. Organisms recovered from the "cystic gland" are not a fair criterion on which to base an assumption of existing infection in the gall bladder.

NEGRI BODIES: Stain for, Jordan, J. H. and Heather, A. H. Stain Tech. 4: 121, 1929.

Fixation:

1. Zenker's solution four hours at 37° C. or Dominici's three hours.
2. 70 per cent alcohol, twelve to eighteen hours at room temperature.
3. 80 per cent alcohol, about five to six hours.
4. 90 per cent alcohol, about four to six hours.
5. Absolute alcohol about sixteen hours.
6. Ether and absolute alcohol aa, about eight hours.
7. Sixteen to twenty-four hours in the following mixture: celloidin 1 g., methyl salycilate 25 c.c., absolute alcohol 25 c.c., ether 25 c.c.
8. Chloroform and paraffin, two to three hours.
9. Paraffin, two hours.
10. Paraffin, one to one and one-half hours.
11. Embed.

Staining:

1. Cut sections 4 to 5 micron.
2. Bring section to water and cover with Lugol's iodine for ten minutes.
3. Decolorize with a 2 per cent sodium thiosulphate (hypo).
4. Wash thoroughly with water.
5. Cover with a mixture of equal parts of 0.5 per cent phloxine and 1 per cent eosin Y (National Aniline brand) and leave for fifteen minutes.
6. Wash with water and stain two to five minutes in 0.1 per cent azure B (National Aniline).
7. Wash with 96 per cent alcohol and decolorize in a mixture of 2 parts absolute alcohol with 1 part clove oil, ordinarily for not more than one-half to one minute.
8. Dehydrate rapidly, clear, and mount in Yucatan Elemi.

OCCULT BLOOD: A Modification of The Strzyzowski Reaction. Oustinoff, P. V. Ann. de Med. Leg. **9**: 477, 1929.

Glacial acetic acid	1 c.c.
Gum arabic (25 per cent)	1 c.c.
or	
Glycerin	1 c.c.
Hydriotic acid	2 to 3 drops

This reagent can be kept for two to three weeks.

A small fragment of the suspected substance, which should be entirely dried, is placed on a glass slide and is covered with a cover glass. A few drops of the reagent are then added. The preparation is heated until it boils for ten to twenty seconds, more reagent should be added. The crystals thus formed are small and are rhombic in shape and are black prisms of hematine iodide.

ALBUMIN: A New Test for: Rose, C. Indian M. Gaz. **64**: 17, 1929.

The reagent used for the new test is a saturated solution of saccharin in water prepared by boiling saccharin in distilled water until no more saccharin is dissolved. Allowing the solution to cool, the next step is to filter it and preserve the clear solution in a stoppered phial. On standing for some time, a few crystals of saccharin may separate out and settle at the bottom; these may be allowed to remain.

The test may be performed in the same way as one does Heller's test with nitric acid. It is best to take a long and narrow test tube, about one-sixth full of clear urine, and hold in a slanting position. Now slowly and carefully allow the reagent to run along the side of the test tube by means of a pipette; it will settle at the bottom. If albumin is present in the urine, even in traces, a sharply defined white ring will slowly form at the junction of the two layers of fluids, best seen when held against a dark background. The ring does not disappear on the application of heat. Strong nitric and picric acids behave in a similar way with albumin in the urine; and with regard to delicacy, all tests are probably of equal merit, but there are certain advantages which the saturated solution of saccharin possesses over the other tests.

The new reagent (saccharin) possesses several advantages over nitric acid in that:

1. It does not precipitate mucin.

2. It does not precipitate urea.

3. It does not precipitate urates unless these are present in the urine in considerable excess.

WASSERMANN REACTION: As a Routine Test In Hospital Practice, Nickson, D. H. and Leibly, F. J. Arch. Dermat. & Syph. **19**: 738, 1929.

In a total of 17,132 consecutive Wassermann tests on adults, the authors found an average of 1.9 per cent of the hospital patients to be syphilitic. The diagnosis was made by the Wassermann test in 248, or 74.2 per cent, of the 334 cases, leaving 25.8 per cent in which the diagnosis was made by the physician or surgeon in charge, before the Wassermann test was done.

From the foregoing facts it may be concluded that:

1. Routine Wassermann reactions in hospitals are not only justifiable, but necessary for the diagnosis in three of every four syphilitic patients admitted.

2. The percentage of syphilitic patients admitted to the Swedish Hospital has been found to be considerably less than that in other general hospitals.

3. Syphilitic men present themselves in hospitals, in a two to one ratio, compared to women.

4. Women in hospitals show the presence of syphilis, on an average, ten years earlier than men.

5. It is approximately 10 per cent easier to diagnose syphilis in men than in women on a basis of history and physical observations.

6. The number of unmarried men with syphilis is high compared to the number of married men who have it, and an unmarried man of middle age is more likely to be syphilitic than a married man of the same age.

7. The mortality rate for syphilitic infants in this hospital was 40 per cent.

FLAGELLA: A Method for Staining, Craigie, J. Brit. J. Exper. Path. **9**: 55, 1928.

Suspensions of twenty-four hour cultures are fixed in formal (physiological saline, or buffer solution at P_H 7, containing 2 per cent formalin). Leave in fixative one hour or overnight. Glassware is cleaned in biehromate-sulphuric-acid. Dilute formolized suspensions in distilled water and spread a small loopful over a cleaned slide. Dry at 37° C. Heat at 90 to 100° for five minutes or longer. Place in distilled water five minutes, then wash in distilled water. Dry and heat again at 90 to 100°. *Mordanting:* Use Zettnow's mordant (10 gm. tannic acid, 200 c.c. distilled water, heated to about 60° and 30 c.c. of 5 per cent aqueous tartar emetic added slowly). Flood slide with mordant and heat five to ten minutes at 90 to 100° F.; watch constantly to prevent drying at edges by adding more mordant as required. Wash under tap, then in distilled water. *Silvering:* Dissolve 1 gm. silver sulphate in 250 c.c. distilled water, and to 20 c.c. of the solution add mono-ethylamine solution (33 per cent B. D. H.) until opacity just clears up. Flood slide with this and warm till steaming begins, holding at mild heat till preparation turns brown or black, but no longer. Wash under tap, then in distilled water. *Gold-toning:* Add 20 drops of 1 per cent gold chloride solution to 20 c.c. distilled water. Immerse slides in this for thirty minutes or longer. Wash and dry.

TUBERCLE BACILLUS: Rapid Staining of, Gaussen, C. Compt. rend. Soc. Biol. **97**: 1657, 1927.

This method stains Koch's bacillus, other organisms, and cells in sputum and is useful for pathologic serous exudates, cephalic-spinal fluid and urine. Make thin film on slide dry at 37°, fix in ether-alcohol, place on hot plate and stain five minutes in Ziehl's solution, made up by triturating in a mortar 1 gr. basic fuchsin, 5 gr. phenol, and 10 c.c. 95 per cent alcohol; add 90 c.c. distilled water and filter. After staining wash in running water, dry, stain for four minutes in a differentiating stain made up as follows:

Dissolve separately (warm) in round bottom flasks:

1. Two and five-tenths gm. Methylene blue, 150 c.c. 80 per cent alcohol, 3 gm. lactic acid;

2. Two gm. orange G, 100 c.c. 80 per cent alcohol, 2 gm. lactic acid;

Put aside 50 c.c. of the blue solution to cool. Mix the rest of blue with orange, heat again, cool, filter. Heat precipitate remaining on filter with 30 c.c. 80 per cent alcohol. Cool, filter, mix two filtrates and add the 50 c.c. of blue solution that has been put aside, filter.

Nuclei are stained violet, acidophil protoplasm yellow, basophilic blue, eosinophil granulations greenish, mucus violet, sero-albuminous exudates yellow, microorganisms blue-green against yellow cytoplasm; Spirillae are gray, diplococci deep violet red, common acid resisting organisms deep maroon. Koch bacillus bright red.

TISSUE STAIN: Preparation of Muci-Carmine, Southgate, H. W. J. Path. & Bact. **30**: 729, 1927.

Precise directions for preparing Mayer's muci-carmine which gave constant results are given. One gram of carmine, 1 gr. of dry powdered aluminum hydroxide 100 c.c. of 50 per cent alcohol, 0.5 gm. of powdered anhydrous albuminum chloride are placed in a 500 c.c. flask which is placed on a boiling water-bath with frequent shaking. The mixture is boiled exactly two and one-half minutes, cooled under the tap and filtered. For staining dilute

1 c.c. of this solution with 9 c.c. of distilled water and stain sections fifteen to twenty minutes. Rinse with distilled water then treat with alcohol, xylol and balsam. This stain colors mucin deeply.

CARCINOMA: Rapid Histologic Diagnosis, Dengler, R. Zentralbl. f. Gynäk. Leipzig 53: 457, 1929.

A piece of the suspected tissue is removed with a pair of fine forceps or a sharp instrument and placed in one drop of 0.9 per cent sodium chloride solution on a slide; it is then torn into shreds with two dissecting needles, covered with a cover glass and examined under a microscope. After cutting down the light and turning to the highest magnification, one determines whether or not the preparation is sufficiently transparent; it is, a drop of 1 per cent acetic acid is placed at one edge of the cover glass. In a short time the microscopic picture becomes clear; the nuclei become distinctly visible, particularly if the diaphragm is closed still more. If one finds in the preparation an area which is sufficiently transparent and which contains almost isolated cells lying side by side, one may begin the counting. The author divides the cells into seven classes according to the size of their nuclei, ranging from Class 1 with the smallest nuclei to Class 7 with the largest nuclei. Each higher class consists of cells whose nuclei are two, three, four, five, six or seven times larger than the smallest nuclei in the tissue. This simplified technic is possible because the size of the nucleus may be taken as an index of the malignancy of a cell. A differential nuclear count of several fields containing the smallest and the largest nuclei should be made in order to get a truly representative count. Erythrocytes, lymphocytes, phagocytes, leucocytes, fibroblasts, and giant cells are readily recognized and are not counted. The result of the count is expressed by the quotients of the count for the various size classes by the total numbers of cells counted. When fifty cells are counted it lies between 3/50 and 7/50, or 0.06 and 0.14; in cancer it is always more than 0.1, usually 0.14. In all of 100 cases in which this method of diagnosis was used, the results were confirmed by the histologic examination of the specimen removed at operation.

SPUTUM STUDIES IN CHILDREN: Direct Laryngoscopy As a Method for Cultural Studies of Pulmonary Secretions In Infants and Children, Goldman, I. B. Am. J. Dis. Child. 38: 47, 1929.

Attention is called to the utilization of the direct laryngoscope for obtaining for cultural and other examinations, sputum or laryngeal and tracheal secretions from infants and children.

SICKLE-CELL ANEMIA: In a Greek Family, Cooley, T. B. and Lee P. Am. J. Dis. Child. 38: 103, 1929.

The authors report the occurrence of this condition in a Greek family thus indicating that it is not, as has been thought, peculiar to the negro race. They suggest that sickle cells should be looked for in all hemolytic anemias.

OTITIS MEDIA: In Infancy: Diagnosis By Means of Cultures Taken From the Middle Ear, O'Donnell, W. S. and Myers, C. Am. J. Dis. Child. 38: 49, 1929.

The contents of the middle ear are obtained by means of a specially made needle, 19 gauge, 3½ inches (8.9 cm.) long. To the needle, a glass observation tube (glass window) is added. A piece of soft rubber tubing, 3 inches (7.6 cm.) long is placed between the observation tube and a glass mouthpiece. With this apparatus, the contents of the middle ear can be withdrawn by aspiration. Before the apparatus is used, it is sterilized by autoclaving in a test tube with a cotton stopper.

The child is wrapped in a blanket and placed on an examining table. The head is held quiet by an assistant. The cerumen and the contents of the aural canal are carefully

removed. Absolute alcohol is then dropped into the canal. Next, sterile cotton is inserted and removed after five minutes. A sterile speculum attached to an electric auriscope is placed in the canal. The needle is inserted into the speculum and through the drum. By suction with the mouth, the contents of the middle ear are withdrawn into the observation tube. The material obtained is immediately transferred to a 1 c.c. of sterile physiologic sodium chloride solution in a small test tube.

Cultures are made from the suspension in physiologic sodium chloride solution. The material is placed in each of two tubes of veal infusion pneumococcus broth, P_H 7.4. In addition streaks are made with a platinum wire on a beef extract, agar blood plate, P_H 7.8. At the same time, a smear of the suspension is made, and examined with gram's stain.

Observations are made before the needle is inserted as to the appearance of the drum, its color and the amount and position of the bulging. This is done in order to ascertain whether the organism cultured from the contents of the middle ear is associated with any characteristic changes in the tympanic membrane.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Biological Stains**

PROBABLY few volumes of similar size will prove more useful to the laboratory worker than this present work.

The outgrowth of the work done by the Commission for the Standardization of Biological Stains, this new edition, much enlarged and comprehensively reviewed, contains a wealth of information. Many of the outstanding staining methods are presented together with numerous useful tables.

The reviewer knows of no other source where this extremely useful information can be found without an exhaustive search of the literature.

The Nervous Child†

THE successive editions of this book have always been welcome, especially to pediatricians and internists, and this fourth edition has been further enhanced by an additional chapter on the "Underlying Disturbances of Metabolism in the Nervous Child," which deals with that more or less baffling group of infants and children who possess that vague and indefinable background referred to by the German school as "Exudative Diathesis." This class embodies eczema, tetany, croup, convulsions, urticaria and asthma, and no study of a nervous child can be considered complete which does not attempt an analysis of this aspect of the problem. This grouping is usually designated by British writers as the "lymphatic type," while the antithesis is the "acid type."

This book can be recommended to that particular class of parents who are the sometimes proud possessors of the negative type of child, and especially the type of infant or child with a neuropathic background. Of extreme value and practicability is the chapter dealing with poor appetite, which the author portrays skillfully and intelligently as one of the perversities of the recalcitrant eater. Marked emphasis is laid upon environment and surcharged atmosphere, "healthy inattention," and stubbornness greater than that possessed by the child, are some of the happy and successful solutions offered.

This small book of 16 chapters, with illustrations, consists of 236 pages, is clearly and concisely written in an interesting manner, almost novel-like in character, as that great pediatric clinician, Dr. Cameron, can portray. This book since its first edition in 1919 is constantly quoted as embodying and exemplifying the practical processes successfully proved by years of experience. This small volume should find a place on every physician's desk as a medium to aid in imparting proper understanding and suggestions to parents, especially those with obstreperous offspring. There are a number of excellent photographs depicting "types" of children, particularly emphasizing the "status catarrhalis" and "postural defective." It would be well worth Dr. Cameron's efforts to present such a book for lay reading.

*Biological Stains. A Handbook on the Nature and Uses of the Dyes Employed in the Biological Laboratory. By H. J. Conn. Second Edition. Cloth, 224 pages. The Commission on Standardization of Biological Stains, Geneva, N. Y.

†The Nervous Child. By H. C. Cameron, M.A., M.D. (Cantab), F.R.C.P. (Lond.) Physician-in-charge of the Children's Dept., Guy's Hospital. Fourth Edition, Oxford Press.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

*Outline of Bacteriology**

THIS small volume is practically a quiz compend for the use of students of oral hygiene, and embodies the material presented by the author to his classes in Columbia University.

Elementary in character, it should prove useful to those to whom it is addressed.

Fundamentals of Pathology†

THIS little volume is intended primarily for dentists and students of oral hygiene and is based upon lectures delivered by the author in the School of Oral and Dental Surgery, Columbia University.

It is, of necessity, succinct and compact and as such constitutes an effective quiz compend very useful to those to whom it is addressed.

Memoranda of Toxicology‡

THE second edition of this well-known little handbook bears evidence of comprehensive revision with the inclusion of much new material concerned not only with newer methods for the treatment of poisoning, but also the newer sources of poisoning, such as terraethyl lead, barium salts, and asphyxiating gases.

The book contains a wealth of authoritative material within a small compass and should be exceedingly useful to the student and practitioner.

Diseases Transmitted from Animals to Man§

THAT disease may be interchanged between man and animals is a matter of general knowledge, but exact information as to *what* diseases, and exactly how they are transmitted as well as the effective means for the prevention and control of such infections is not as generally disseminated as it might be possibly because this information is to be found in numerous and sometimes out of the way places.

In compiling this book, therefore, the author has done no small service to the clinician, the epidemiologist, and the pathologist.

The book is divided into 5 sections.

Section I covers Diseases of Domestic Animals and Birds; Section II, Rodent Affections; Section III, Human Diseases Spread by Animals; Section IV, Animals as Passive Carriers of Disease Organisms; and Section V is devoted to a review of the Rôle Played by Each Animal in the Spread of Disease.

There is also an alphabetical index of authors quoted or referred to in the text.

At the end of each chapter the salient features of the disease in question are reviewed in a series of short, crisp paragraphs.

The book is a useful and valuable addition to medical literature deserving of wide circulation.

It can be highly commended to all who are interested in the problem of disease.

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*Outline of Bacteriology. By H. A. Bartels. Lecturer on Bacteriology, School of Oral Hygiene, Columbia University. Cloth, 128 pages, 48 figures. W. A. Broder, New York.

†Fundamentals of Pathology. By Joseph Schroff, Assoc. Prof. Oral Pathology, Columbia University. Cloth, 119 pages, 40 figures. W. A. Broder, New York.

‡Memoranda of Toxicology. By M. Trufper, Consulting Toxicologist, Graduate School, Univ. of Penna. Second edition. Leather, 214 pages, P. Blakiston's Son & Co., Philadelphia.

§Diseases Transmitted from Animals to Man. By T. G. Hull, Chief Bacteriologist, Illinois Dept. Health, etc. Cloth, 350 pages, 29 illustrations. C. C. Thomas, Springfield, Ill.

*Recent Advances in Preventive Medicine**

PREVENTIVE medicine no longer means merely what is now called sanitation but embraces not only every field of medicine but sociologic and physiologic aspects as well.

In the present volume is given a fairly comprehensive and diverse survey of the recent advances in this field.

Among the subjects considered are: eugenics, maternal mortality, wastage of young life, childhood, milk and milk-borne diseases, recent advances in our knowledge of vitamins, atmospheric conditions, hygiene in industry, and active immunization.

The discussion of all of these subjects is drawn from a thorough survey of the literature, a list of the references utilized being appended to each chapter.

The volume should be of interest and value to all who are concerned with the problem of disease.

Human Helminthology†

THERE are few subjects in medicine concerning which information is less generally disseminated than helminthology, possibly because of the irregular geographic distribution of the diseases in which it is concerned, and perhaps because of the special training required for its competent study.

In this present volume Dr. Faust, whose experience well qualifies him for the task, presents a comprehensive text embodying all the important steps in its recent developments.

Section I of the book covers clearly the fundamentals of the subject including the literature; Section II discusses systematically the flatworms; and Section III the roundworms.

Section IV is of great interest and value as it presents the laboratory problems concerned with the study of helminthology.

The newer methods are clearly described and, when necessary, amply illustrated. Chapter XXXII, The Identification and Differential Diagnosis of Helminth Parasites and Their Eggs, will prove exceedingly useful in those laboratories where this kind of work is relatively infrequently done.

The final chapter concerns the intermediate and reservoir hosts involved.

All in all the volume is a credit to both author and publisher and seems destined to become a standard text. It can be highly recommended to both physician and laboratory worker.

Tularemia‡

THE gradual recognition of tularemia as an endemic infection of marked economic importance makes this volume most timely.

Its history, pathology, diagnosis, and treatment are comprehensively discussed by Dr. Simpson whose work in connection with the disease is well known.

In view of the fact that many cases have been overlooked in the past, the chapter on the clinical manifestations of tularemia will be most useful.

The illustrations, which are numerous, are excellent in every way.

The volume can be recommended without reserve and should be in the hands of every practitioner.

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*Recent Advances in Preventive Medicine. By J. F. C. Haslam, Assistant Director, Bureau of Hygiene and Tropical Diseases, etc. With A Chapter On the Vitamins, by S. J. Cowell, Professor of Dietetics, Univ. of London. Cloth, 328 pages, 30 illustrations. P. Blakiston's Son & Co., Philadelphia.

†Human Helminthology. A Manual for Clinicians, Sanitarians and Medical Zoologists. By Ernest Carroll Faust, Ph.D., Professor of Parasitology in the College of Medicine of Tulane University, New Orleans, La. Octavo, 616 pages, illustrated with 297 engravings, cloth. Lea and Febiger, Philadelphia.

‡Tularemia, History, Pathology, Diagnosis and Treatment. By W. M. Simpson. Cloth, 162 pages, 53 figures, 2 colored plates. P. B. Hoeber, New York.

*Arthritis**

CHRONIC nontuberculous arthritis is receiving considerable attention. Literature upon this subject is rapidly accumulating. Obviously, it is becoming more and more difficult for the practitioner to choose his reading material from the recent volumes on the subject. The average physician has not time to speculate upon theory. He must have his feet firmly set upon the foundation of basic principles.

Fisher has built his book upon a clear insight into joint physiology, and has elaborated his contribution along physiologic lines. His treatment of joint pathology is somewhat different from that of similar works. The effort has been made to give more space to description of early joint changes without neglecting the usual classical pictures of advanced pathology. This tends to clear the misunderstanding that has arisen between the pathologist who sees only terminal changes and the clinician who most frequently attacks the disease in its incipiency.

Therapy is adequately treated under three classifications, first, medical treatment; second, physiotherapy, manipulation, and other orthopedic measures; and third, surgical operations in chronic arthritis.

The volume is profusely illustrated.

Wiggers' Electrocardiography†

WHEN Wiggers writes on the heart one expects it to be good. As usual this time it is. The volume is based upon the author's lectures to his students at Western Reserve University on the heart and particularly upon the use of the electrograph and the interpretation of the electrocardiogram. The work is divided into three sections. The first section deals with apparatus, technic, the physics of electrocardiography, and describes and discusses the various instruments on the market. The author preserves a strict impartiality as to the merits of this or that machine.

The second section describes and discusses the normal electrocardiogram and the significance of normal and abnormal deflexions as observed in clinical work.

The third section is decidedly an innovation and is the portion of the book that makes it different from other volumes on electrocardiography. In it the author presents a series of abnormal tracings as unknowns and proceeds forthwith to analyze each tracing ending up each time with a diagnosis, discussion and rather comprehensive review of treatment. It reminds one somewhat of Cabot's Case Reports which have become so deservedly well known.

The unknowns have been selected and arranged so as to cover all of the usual and most of the unusual types of tracing that one is likely to encounter. In general there is only one example of each. While the discussion is consequently complete we nevertheless hope and anticipate that in the next edition Dr. Wiggers will include a large number of additional unknowns with notes on their interpretation so that the student may have further opportunity for developing his skill in interpretation.

The volume is primarily a textbook for students and a reference work for the internist, who wishes to acquire reasonable skill in electrocardiographic interpretation. As such the reviewer feels that it is the best work on the subject that has come out since Lewis' *Clinical Electrocardiography*.

The Immunology of Parasitic Infections‡

THE author has undertaken to compile the scattered observations on this subject, the literature of which is surprisingly large. As a consequence the volume will be of great interest to those interested in clinical and experimental serology.

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*Chronic (Nontuberculous) Arthritis. By A. G. Timbrell Fisher M.C., F.R.C.S.(Eng.) Joint Lecturer on Operative Surgery, London School of Clinical Medicine. Cloth, 186 illustrations, pages 220. The Macmillan Company, New York, 1929.

†Principles and Practice of Electrocardiography. By Carl J. Wiggers, M.D. Professor of Physiology in the School of Medicine of Western Reserve University, Cleveland, Ohio. With 61 Illustrations. Cloth, pages 226. The C. V. Mosby Company, St. Louis, 1929.

‡The Immunology of Parasitic Infections. By William H. Taliaferro, Ph.D. Professor of Parasitology. The University of Chicago. Cloth, 414 pages. The Century Company, New York, London, 1929.

It will be of interest to those who have not made a special study of the subject, to know that there is a fairly promising flocculation reaction for kala-azar and a reliable complement-fixation reaction for horse trypanosomiasis. There is evidence of antibody formation in malaria but so far no serologic test has been perfected to the point where it can be of practical diagnostic aid. The handicap in preparing a suitable test antigen lies in obtaining sufficient malaria organisms free from host protein. So far there is no satisfactory serologic test for coccidiosis. The results in schistosomiasis are promising but so far inconclusive. In hydatid disease the intradermal test for echinococcus infection is very promising. There is promise of a satisfactory precipitin test for trichinosis after infection has been well established, and for an intradermal test for early infection. Although in no way standardized, there seems considerable promise that serologic tests may be developed in amebiasis. The Wassermann test for syphilis, with the more modern methods, is not positive in various parasitic infections of man unless syphilis or yaws is present. On the other hand it may be positive with the serums of rabbits infected with trypanosomes.

There is evidence that in experimental trypanosomiasis the animal develops a trypanolytic immunity which increases and decreases periodically in the infected animal. The serum of animals which have recovered from this disease appears to carry immune bodies which may be used for passive immunization.

Eosinophilia is characteristic of many of the protozoan invasions. Eosinophilotactic substances have been obtained from many worms and our efficacy in producing eosinophilia is largely dependent upon previous sensitization. The reticuloendothelial system, presumably the source of immune bodies, is known to be parasitized by the Leishmania and certain of the sporozoa which by their invasion appear to inhibit or decrease antibody production. However the reticulo-endothelial system is the chief defense of the body in malaria, is responsible for the formation of the humoral antibody which inhibits cell division of T. Lewisi, and it is important in the activation of the trypanocidal property of normal human serum and of drugs.

The author makes one very pertinent statement which we cannot refrain from quoting. "There is a tendency to treat antibodies as if they were definitely known chemical complexes, when, as a matter of fact, they are known only as properties or manifestations of antiserum, and are postulated only in terms of what the antiserums will do under certain conditions, just as enzymes are known by what they do rather than by what they are."

*Mammalian Physiology**

A BEAUTIFULLY produced laboratory manual for the use of students in experimental physiology, well illustrated with kymographic tracings, line drawings, and color sketches. Marginal headings facilitate orientation, and annotations at the end of each of the twenty-two exercises provides discussion and historical references.

This volume should be in the hands of every teacher of physiology. Some will probably use it as a laboratory manual.

Practical Psychology and Psychiatry†

A TEXTBOOK for nurses and medical students and reference manual for physicians. The first portion touches the high lights of psychology and the second portion presents an excellent recapitulation of clinical psychiatry. The average physician while not a psychiatrist should have a more intimate understanding of diseases of the psyche than he usually does. This work will enable him to brush up on the high lights without going into unnecessary detail. While the psychiatrist would find it rather elementary we can recommend it most highly for the purposes for which it was intended.

*Mammalian Physiology. A Course of Practical Exercises. A New Edition. By E. G. T. Liddell, D. M., Fellow of Trinity College, Oxford, and Sir Charles Sherrington, O.M., M.D., D.Sc.(Cantab.), F.R.S. Waynflete, Professor of Physiology in the University of Oxford. Cloth, pages 162. Oxford University Press, American Branch, New York, 1929.

†Practical Psychology and Psychiatry. For Use in Training Schools for Attendants and Nurses and in Medical Classes, and as a Ready Reference for the Practitioner. By C. B. Burr, M.D. Sixth edition revised and enlarged, with illustrations, pages 378, cloth. Davis, Philadelphia.

*Bronchial Asthma**

NOW that developments in the field of allergy have awakened interest in the possibility of relieving bronchial asthma and allied conditions the monographic literature on this subject is becoming more abundant. The greater part of these discussions are based on considerations of protein sensitization.

Alexander has gone back to fundamentals. His work might be termed briefly the structural and functional pathology of bronchial asthma. After a short historic review he presents in detail the known facts of the anatomy and innervation of the bronchi and air sacs. This is followed by a review of the theories of the mechanism of the asthmatic paroxysm and the factors which initiate an attack. The next chapter deals with the pathology of asthma, and the following with its immunologic aspects. There follow chapters on the clinical features, complications, treatment, and prophylaxis. The author's discussion of the effect of asthma on the heart and circulation is especially interesting. The evidence today would indicate that the asthmatic paroxysm itself except for a temporary partial asphyxia has little or no damaging effect upon the heart. Instead of causing cardiac dilatation, if anything the heart is smaller during the attack. Even at postmortem in cases of long-standing asthma and emphysema no constant cardiac lesion is to be found.

On the other hand, in chronic pulmonary emphysema there develops some hypertrophy and dilatation of the right ventricle. This presumably is the mechanical result of increased pressure in the pulmonary artery due to partial obstruction in the pulmonary circuit. Emphysema accompanying bronchial asthma does not appear to be associated with this type of structural change.

On the other hand the peripheral venous pressure is markedly increased in the asthmatic attack. It is possible that this partial venous stasis, especially when associated with emphysema, may account for the subcutaneous edema occasionally seen in advanced cases of bronchial asthma. This edema together with the dyspnea upon exertion and the cyanosis resulting from emphysema simulates the picture of cardiac decompensation and has given rise in the past to the misconception that bronchial asthma is likely to produce congestive heart failure.

Alexander stresses the value of sputum examination. Too often nowadays this is overlooked, and yet it may contain valuable information.

In a short appendix the skin tests are described and discussed.

Alexander's monograph covers a field of the subject of asthma which is rather neglected in most of the other recent monographs. It should be in the hands of those who are interested in the subject.

Goiter Prevention and Thyroid Protection†

A SEMIPOPULAR presentation of diseases of the thyroid gland, which presupposes no previous acquaintanceship with the subject on the part of the reader. Like many such discussions of a single subject it places perhaps a little too much emphasis on the thyroid to the exclusion of the rest of the body. It is a question whether some harm is not done by placing this type of book in the hands of the laity, for lay persons who read these works are usually nervous, introspectives and who possess no stabilizing background of general medical perspective.

For the physician who has never made any special study of the diseases of the thyroid gland and who wants to have an easily assimilable work on the subject this volume should be of some interest.

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*Bronchial Asthma. Its Diagnosis and Treatment. By Harry L. Alexander, A.B., M.D. Associate Professor of Medicine in the Washington University Medical School, St. Louis, Mo.; Associate Physician to the Barnes Hospital, St. Louis, Mo. Cloth, pages 171, illustrated. Lea & Febiger, Philadelphia, 1928.

†Goiter Prevention and Thyroid Protection. By Israel Bram, M.D. Author of "Goiter, Nonsurgical Types and Treatment"; Medical Director, Bram Goiter Institute, Upland, Pa.; formerly Instructor in Clinical Medicine, Jefferson Medical College, Philadelphia; Fellow of the American Medical Association, the Association for the Study of Internal Secretions, the American Association for the Study of Goiter, the American Medical Editors' Association, etc. Illustrated, cloth, pages 327. F. A. Davis Company, Philadelphia, 1928.

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DEAN LEWIS, M.D. - - - - - - - BALTIMORE

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EDITORIALS

Barbital Poisoning

ONE of the more common types of poisoning is that with diethylbarbituric acid (veronal). This drug is pretty generally known to the laity and many cases of habit-formation from its use have been reported. The acute symptoms are fairly characteristic but may be confused with those of other hypnotics, particularly those of the barbituric acid series. The pupils are constricted (as also often occurs in acute chloral poisoning and to a less extent with codeine) and may thus lead the physician to suspect morphine poisoning.

Much work, both clinical and experimental, has been done in the past in the matter of investigating and treating cases of acute barbital poisoning. The smallest fatal dose for man has been found to vary between 0.6 and 10 gm., but the average fatal dose for man is probably about 50 gr. (3.3 gm.). This wide variation in the size of the fatal dose has been emphasized in many clinical reports, but the explanation of this phenomenon has been somewhat obscure. Consequently it has been exceedingly difficult to properly evaluate the results of any given form of medical treatment.

In a splendid paper recently published by Gower and Tatum[1] much new light has been thrown on the variations to be found in veronal poisoning. Over a period of two years these investigators have carried on experiments under a variety of conditions and they have found that in carefully controlled experiments most dogs recover from a dose of from 200 to 250 mg. per kilogram of body weight given intravenously. On account of its solubility they have used the sodium salt of veronal (medinal). Doses of 400 mg. per kilogram were fatal within one to five days in about 70 per cent of the cases. Animals which recover from a dose of 400 mg. per kilogram are likely to recover again if the same dose be repeated at a later date. One such animal recovered from this dose on five occasions under various treatments. On the other hand 200 mg. per kilogram produced death in some cases. Similar variations among animals, not only of the same but of different species as well, have also been noted by Nielsen, Higgins and Spruth[2] and by Eddy.[3] It is found at autopsy that there is a considerable variation in the amount of the drug present in the different organs and tissues of the body, thus indicating a selective absorption. For example, the central nervous system and the red corpuscles both contain relatively high concentrations of the drug in comparison with other tissues.

Excretion occurs almost entirely by the kidneys. It seems probable that some portion of the drug is destroyed in the body for only about 50 to 90 per cent can be obtained from the urine. The excretion is a slow process and this is of especial importance from a clinical standpoint. The cause of death appears to be respiratory failure, or effects secondary to prolonged coma such as bronchopneumonia, impairment of the circulation with pulmonary edema and cyanosis, lack of proper nourishment, etc. Jacobj and Roemer[4] believed that veronal possessed a specific toxic action on the smaller blood vessels causing capillary dilatation and a fall of blood pressure similar to that under acute arsenic poisoning. The heart becomes weakened, there is a fall in temperature, the reflexes are sometimes increased and very rarely there may be convulsions, the respiration becomes slow and shallow, the urine is diminished or totally suppressed, and necrosis of the kidney epithelium has been described. Dermatitis not infrequently occurs and the pupils may often show rhythmic variations.

The treatment of barbital poisoning in the past has included supporting measures such as heat, liquid food, heart stimulants and removal of the unabsorbed drug from the gastrointestinal tract. Fluids and drugs have been given to stimulate renal activity and bleeding or the removal of cerebrospinal fluid has been advised. Stimulants have been given to overcome the depression. Clinically it is difficult to determine which of these measures is more likely to be of most help to the patient because of the marked variations in resistance to the poison which different patients may exhibit. But from the experimental evidence Gower and Tatum believe that the surest means of helping the patient is to secure as marked and as prolonged an increase in urinary secretion as possible. For these investigators found that, in dogs, tolerant animals were those that had a high rate of urinary excretion of the drug, while animals that remained in a critical condition for several

days after relatively small doses and recovered slowly were animals possessing a low rate of renal elimination of the drug. They, therefore, believe that tolerance for barbital is dependent directly on the relative ability of the animal (or man) to excrete the drug in the urine. They found, however, in one of their experiments, that when diuretic procedures (rapid injection of glucose solution) were employed, the fluid of the urine might increase twenty times but that the rate of excretion of the veronal was not even so much as doubled. For while a high urinary output tends to increase the amount of drug eliminated for a time, in the course of an eight- or ten-hour period the rate falls off so that the total drug output is no greater than would be secured by a lower volume secretion of urine.

The solubility of barbital in water is limited, however, so that the water volume should exceed that required to maintain the barbital in solution.

With reference to bleeding the authors conclude that the actual quantity of barbital removed in withdrawn blood is of no practical significance in comparison to a high rate of renal excretion. Bleeding without return of fluid to the circulation uniformly causes a drop in the rate of urine flow and of barbital excretion but this drop in excretion may be avoided if the volume of the withdrawn blood is immediately replaced by the injection of physiologic salt solution. In dogs saline infusion following bleeding seemed less likely to cause retention of fluid in the tissues with resulting edema than an infusion alone without the preparatory blood withdrawal. But these measures are scarcely to be recommended, and the chief aim of therapy in acute barbital poisoning should be directed toward the maintenance of optimum renal excretion over a long period of time, and more can be hoped for from consistent moderate diuresis than from extreme diuretic measures. It is desirable that the urine be alkaline in order to favor the solubility of the barbital.

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—D. E. J.

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News and Notes

In regard to the work of the Research Committee on the problem of Undulant Fever, Dr. A. S. Giordano has kindly offered to furnish the material for doing the skin tests put up in vials for distribution to the Members of the American Society of Clinical Pathologists throughout the country. We would like very much to have as many Members as possible obtain this material, make the tests and report the results to Dr. A. G. Foord, Chairman of the Research Committee, Buffalo General Hospital, Buffalo, New York.

To secure this material, communicate with Dr. A. S. Giordano, South Bend Medical Laboratory, South Bend, Indiana.

At the Ninth Annual Convention in Detroit this June, the American Society of Clinical Pathologists will have more space available for Scientific Exhibits than ever before. We are therefore anxious to have as many of our Fellows as possible present exhibits of interest to the Membership. This is fast becoming a very valuable feature of our Conventions and to stimulate participation in this event there are offered two prizes consisting of a Gold and a Silver Medal to be awarded to the two Members of the American Society of Clinical Pathologists presenting the best scientific exhibit. You may communicate with the Secretary, 256 Metropolitan Building, Denver, Colorado, or Dr. C. I. Owen, Grace Hospital, Detroit, Michigan, to reserve space for this exhibit.